Antibody and/or chemokine constructs and their use in immunological disorders

The present invention relates to the use of an antibody and/or chemokine construct which binds to a chemokine receptor for the preparation of a pharmaceutical composition for the elimination of cells which are latently infected with a primate immunodeficiency virus. In addition, the present invention provides for the use of an antibody and/or chemokine construct which binds to a chemokine receptor for the preparation of a pharmaceutical composition for the treatment, prevention and/or alleviation of inflammatory renal diseases, inflammatory bowel diseases, multiple sclerosis, skin diseases, diabetes or transplant rejection. Furthermore, the invention relates to antibody constructs and/or chemokine constructs, in particular to constructs wherein said antibody construct comprises a binding site for chemokine receptor 5 and a binding site for CD3 and wherein said chemokine construct comprises RANTES and a toxin. The invention also describes polynucleotides encoding said antibody- or chemokine constructs, and vectors and hosts comprising said nucleic acid molecules. Additionally, the present invention relates to compositions comprising said antibody constructs, chemokine constructs, polynucleotides, vectors and/or hosts. Preferably said composition is a pharmaceutical composition. Described is also the use of antibody constructs, the chemokine constructs, the polynucleotides, the hosts and/or the vectors for the preparation of a pharmaceutical composition for treating, preventing and/or alleviating an immunological disorder or for eliminating latently infected cells, wherein said cells are infected with a primate immunodeficiency virus, like HIV-1. The present invention also relates to a method for treating, preventing and/or alleviating an immunological disorder or for the elimination cells which are latently infected with a primate immunodeficiency virus. Furthermore, the invention provides for a kit comprising the compounds of the invention.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including any manufacturers specifications, instructions, etc.) are hereby incorporated by reference.

Immunological diseases/disorders, like autoimmune diseases, inflammation disorders as well as infections diseases are not only increasing but represent substantial threats to global health.

For example, in Germany, about 1% of the population suffer from the autoimmune disease rheumatoid arthritis. In addition, there is a number of other rheumatoid diseases also leading to arthritis. Currently, three groups of drugs - non-steroidal antirheumatics, cortisone preparations and second-line agents - and TNFα blocking agents are used for treating inflammatory joint diseases. Up to now, the therapy has focused on the local injection of cortisone preparations in combination with a systemic administration of antiphlogistics or second-line agents.

Non-steroidal antirheumatics have a mild analgetic and anti-inflammatory effect, but they have many side effects when applied frequently (e.g. gastric ulcers, nephroses). In high dosages, cortisone preparations have a strong decongestant and analgetic effect, however leading to a quick relapse after discontinuation of the therapy. Moreover, cortisone preparations cannot stop the destruction process of the joint disease. A long-term therapy with cortisone usually entails severe side effects (infections, Cushing's phenomenon, osteoporosis, parchment-like skin, metabolic and hormonal disorders). The local injection of cortisone also has the essential disadvantage that the activity of the migrated white blood cells is only reduced. As the infiltrating cells are not destroyed, a quick relapse occurs after discontinuation of the therapy. As mentioned above, the same applies to the systemic application. Rarely is an inflammation due to the irritative effect of cortisone crystals aggravated after injection of cortisone. The duration of effect of a cortisone injection varies tremendously and ranges from primary ineffectiveness to a duration of effect of several weeks.

In rheumatology, second-line agents are used to achieve a long-term suppression of the inflammation and a reduction in cortisone preparations. Due to the considerable toxicity (allergies, infections, malignant diseases, renal insufficiency, blood pressure crises, pulmonary diseases) it is necessary for medical specialists to attend closely to the patients. After beginning treatment, no therapeutic effect may be apparent for the first three months. Currently, there are 4 or 5 of such second-line agents at disposal, which are used individually at first or are combined if the therapy is not effective. Mostly, there is hardly anything known about the mode of action of second-line agents. It is not yet entirely clear whether the application of second-line agents can diminish the destruction of the joint. In recent years, a new group of substances has been introduced into the treatment of rheumatoid arthritis, which is based on the blocking of cell signal substances (particularly TNFα) by means of monoclonal antibodies or soluble receptor constructs.

In addition, there are patients that do not respond to currently available therapies. In other cases, the conventional therapy has to be stopped due to intolerable side effects.

A similar situation exists for many other inflammatory and autoimmune diseases like inflammatory renal diseases, inflammatory bowel diseases, multiple sclerosis and transplant rejection, where current treatments have many limitations. For example, agents used in inflammatory and autoimmune diseases include anti-inflammatory and immunosuppressive agents like azathioprine, cyclophosphamide, glucocorticoids like prednisone and corticosteroids; immunosuppressants like cyclosporin A, Tacrolimus (FK506), Sirolimus (Rapamycin); and protein drugs like calcineurin, beta-interferon, anti-TNF alpha monoclonal antibodies (remicade). These agents show general immunomodulating effects and therefore efficacy and side effects profiles can pose severe limitations for the treatment options (Harrison's Principles of Internal Medicine, eds. Fauci et al., 14th edition, McGraw-Hill publisher).

Inflammatory bowel diseases (examples are Crohn's disease, ulcerative colitis) are treated with the anti-inflammatory agents sulfazsalazine (Azulfidine) and glucocorticoids, like prednisone and, in selected cases, with TNF-α blocking agents. In ulcerative

colitis immunosuppressive therapy with drugs such as azathioprine is well established, in severely ill patients the potent immunosuppressive agent cyclosporine is used (Harrison's Principles of Internal Medicine, eds. Fauci et al., 14th edition, McGraw-Hill publisher).

In many cases no sufficient reduction of disease activity is achieved with current drugs, such that even surgical intervention is sometimes necessary.

Inflammatory renal diseases (nephritis) are treated with e.g. glucocorticoids, alkylating agents and/or plasmapheresis. Additional diseases with similar treatment options include systemic lupus erythemtosus (SLE), Sjoegren's syndrome, polymyositis, dermatomyositis, mixed connective tissue disease, antiphospholipidantibody syndrome.

For some of these diseases, few therapeutic options have been available up to now. All these diseases share an inflammatory component. However, the inflammatory component cannot be sufficiently suppressed by the currently available drugs. For some drugs, e.g. alkylating agents a maximal lifetime dose per patient cannot be exceeded.

Transplant rejection is treated using immunosuppressive agents including azathioprine, mycophenolate mofetil, glucocorticoids, cyclosporine, Tacrolimus (FK506), Sirolimus (Rapamycin). A combination of steroids and a low dose of mouse monoclonal antibody OKT3 binding to CD3 on T-cells is used to anergize and deplete T-cells, therapy is continued using immunosuppressants like cyclosporine. Human anti-mouse antibodies I(HAMAs) have common side effects and limit the use of OKT3 (Fauci et al. sic. 2374-2381).

Approaches to treat multiple sclerosis include treatments which effect the overall immune system like anti-inflammatory agents including azathioprine, cyclophosphamide, prednisone, corticosteroids, cyclosporin A, calcineurin, Rapamycin, beta-interferon (Fauci et al. sic. 2415-2419; Wang (2000) j. Immunol. 165, 548-57). In addition, a number of non-specific treatments are administered that may improve the quality of life including physical therapy and psycho-pharmacological agents. None of the treatment

options mentioned above has a curative effect. Even the most promising compound, β-interferon, leads only to a slower disease progression, while exhibiting significant side effects.

Furthermore, human immunodeficiency virus-type 1 (HIV-1), the most common cause of AIDS, has infected more than 50 million individuals (including those who have died), and the rate of new infections is estimated at nearly 6 million per year (AIDS Epidemic Update: December 1999 (UNAIDS, Geneva, 1999), www.unaids.org). Equally disturbing are the uncertainties of the epidemic to come. Although sub-Saharan Africa remains the global epicenter, rates of infection have increased in recent times in the former Soviet Union and parts of south and southeast Asia, including India and China, where literally hundreds of millions of individuals are potentially at risk. In the United States, new waves of infection have been recognized in women, minorities, and younger generations of gay men. Combination antiretroviral therapy has afforded many people clinical relief, but the costs and toxicities of treatment are substantial, and HIV-1 infection remains a fatal disease. Moreover, the vast majority of infected people worldwide do not have access to these agents. Thus, although the demographics (and, in some instances, the natural history) of AIDS have changed, the epidemic is far from over; instead, it is evolving, expanding, and posing ever greater challenges.

Human immunodeficiency virus (HIV) cannot enter human cells unless it first binds to two key molecules on the cell surface, CD4 and a co-receptor. The co-receptor that is initially recognized is CCR5, later in the life cycle of the virus another chemokine receptor CXCR4 becomes the co-receptor for HIV-1 (D'Souza, Nature Med. 2, 1293 (1996); Premack, Nature Med. 2, 1174; Fauci, Nature 384, 529 (1996)). The HIV-1 strains that cause most transmissions of viruses by sexual contact are called M-tropic viruses. These HIV-1 strains (also known as NSI primary viruses) can replicate in primary CD4+ T-cells and macrophages and use the chemokine receptor CCR5 (and, less often, CCR3) as their coreceptor. The T-tropic viruses (sometimes called SI primary) can also replicate in primary CD4+ T-cells but can in addition infect established CD4+ T-cell lines in vitro, which they do via the chemokine receptor CXCR4 (fusin). Many of these T-tropic strains can use CCR5 in addition to CXCR4, and some can en-

ter macrophages via CCR5, at least under certain in vitro conditions (D'Souza, Nature Med. 2, 1293 (1996); Premack, Nature Med. 2, 1174; Fauci, Nature 384, 529 (1996)). Whether other coreceptors contribute to HIV-1 pathogenesis is unresolved, but the existence of another coreceptor for some T-tropic strains can be inferred from in vitro studies. Because M-tropic HIV-1 strains are implicated in about 90% of sexual transmissions of HIV, CCR5 is the predominant coreceptor for the virus in patients; transmission (or systemic establishment) of CXCR4-using (T-tropic) strains is rare (D'Souza, Nature Med. 2, 1293 (1996); Premack, Nature Med. 2, 1174; Fauci, Nature 384, 529 (1996), Paxton, Nature Med. 2, 412 (1996); Liu, Cell 86, 367 (1996); Samson, Nature 382, 722 (1996); Dean, Science 273, 1856 (1996); Huang, Nature Med. 2, 1240 (1996)). However, once SI viruses evolve in vivo (or if they are transmitted), they are especially virulent and cause faster disease progression (D'Souza, Nature Med. 2, 1293 (1996); Premack, Nature Med. 2, 1174; Fauci, Nature 384, 529 (1996), Schuitemaker, J. Virol. 66, 1354 (1992); Connor, J. Virol. 67, 1772 (1993); Richman, J. Infect. Dis. 169, 968 (1994); R. I. Connor et al., J. Exp. Med. 185, 621 (1997); Trkola, Nature 384, 184 (1996)).

The numbers and identity of coreceptor molecules on target cells, and the ability of HIV-1 strains to likely enter cells via the different coreceptors, seem to be critical determinants of disease progression. These factors are major influences on both host- and virus-dependent aspects of HIV-1 infection. For example, a homozygous defect (delta 32) in CCR5 correlates strongly with resistance to HIV-1 infection in vivo and in vitro. Individuals who are heterozygous for a defective CCR5 allele are at best weakly protected against infection and have only a modestly slowed disease progression (Paxton, Nature Med. 2, 412 (1996); Liu, Cell 86, 367 (1996); Samson, Nature 382, 722 (1996); Dean, Science 273, 1856 (1996); Huang et al., Nature Med. 2, 1240 (1996)). However, other factors can influence the level of CCR5 expression on activated CD4+ T-cells and thereby affect the efficiency of HIV-1 infection in vitro (Trkola, Nature 384, 184 (1996); Bleul, Proc. Natl. Acad. Sci. U.S.A. 94, 1925 (1997)). For reasons that are not yet clear, the amount of CCR5 expression on the cell surface (as measured by MIP-1 binding) varies by 20-fold on CD4+ T-cells from individuals with two wild-type CCR5 alleles (Trkola, Nature 384, 184 (1996)) (see figure). Staining with a CCR5-specific

monoclonal antibody indicates a similar large variability (Wu, J. Exp. Med. 186:1373-81 (1997)). Such variation may far outweigh any effect of one defective allele for CCR5. The causes of this variation should be the subject of intensive studies, as they point to controllable factors that could increase resistance to disease.

Most primary, clinical isolates of primate immunodeficiency viruses use the chemokine receptor CCR5 for entry (Feng, Science 272, 872 (1996); Choe, Cell 85, 1135 (1996); Deng, Nature 381, 661 (1996); Dragic et al., Nature 381, p. 667; Doranz, Cell 85, 1149 (1996); Alkhatib, Science 272, 1955 (1996)). For most HIV-1 isolates that are transmitted and that predominate during the early years of infection, CCR5 is an obligate coreceptor, and rare individuals that are genetically deficient in CCR5 expression are relatively resistant to HIV-1 infection (Connor, J. Exp. Med. 185, 621 (1997); Zhang, Nature 383, 768 (1996); Björndal, J. Virol. 71, 7478 (1997); Dean, Science 273, 1856 (1996); Liu, Cell 86, 367 (1996); Paxton, Nature Med. 2, 412 (1996); Samson, Nature 382, 722 (1996)). HIV-1 isolates arising later in the course of infection often use other chemokine receptors, frequently CXCR4, in addition to CCR5. Studies of chimeric envelope glycoproteins demonstrated that the third variable (V3) loop of gp120 is a major determinant of which chemokine receptor is used (see references above and also Cocchi, Nature Med. 2, 1244 (1996); Bieniasz, EMBO J. 16, 2599 (1997); Speck, J. Virol. 71, 7136 (1997)). V3-deleted versions of gp120 do not bind CCR5, even though CD4 binding occurs at wild-type levels. Antibodies to the V3 loop interfere with gp120-CCR5 binding (Trkola, Nature 384, 184 (1996); Wu, Nature 384, 179 (1996); Lapham, Science 274, 602 (1996); Bandres, J. Virol. 72, 2500 (1998); Hill, Science 71, 6296 (1997)). These results support an involvement of the V3 loop in chemokine receptor binding.

Latency of HIV is established very early in the course of an infection, when M-tropic strains predominate. M-tropic strains depend on the presence of CCR5 on the target cell for infection. The importance of CCR5 as an essential co-receptor for M-tropic HIV-1 is emphasized by the fact that individuals lacking CCR5 due to a homozygous 32 basepair deletion (delta32) are highly resistant to HIV-1 infection. In contrast to other markers like CD4, CD25, or CD45RO, CCR5 is only present on a subset of lymphocytes and other cells that are prone to HIV-1 infection (Rottmann (1997) Am J Pathol

151, 1341-1351; Naif (1998) J Virol 72, 830-836; Lee (1999) Proc. Natl Acad. Sci. 96, 5315-5220).

Several approaches have been postulated to eliminate latent infected cells. One strategy is to drive the latently infected to virus production and subsequent cell death. In this context, one approach is IL-2 (TNF-alpha, IL-6) administration in the presence of HAART until the viral reservoir is exhausted (Chun (1998) J. Exp. Med. 188, 83-91; Chun (1999) Nat. Med. 5, 651-655; Stellbrink (1999) Abstracts of the 6th Conference on Retroviruses and Opportunistic Infections (Foundation for Retrovirology and Human Health, Alexandria, VA), abstr. 356. p. 135; Imamichi (1999) Abstracts of the 6th Conference on Retroviruses and Opportunistic Infections (Foundation for Retrovirology and Human Health, Alexandria, VA), abstr. 358, p. 135). These cells are believed to die after activation. Whether the entire pool of latent infected cells can be exhausted is questionable.

Another strategy tried was to specifically kill latently infected cells based on gp-120 expression on the cell surface. Immunotoxins recognizing gp-120 have been proposed but failed for two reasons. The one construct tested in humans was a protein consisting of soluble CD4 linked to Pseudomonas aeroginosa exotoxin A (PE). The clinical results were disappointing due to dose-limiting hepatotoxicity without showing signs of efficacy and the program was terminated (Ashorn (1990) Proc. Natl Acad. Sci 87, 8889-8893; Berger (1998) Proc. Natl Acad. Sci. 95, 11511-11513). The second reason for failure was that latent infected cells do not express viral surface glycoproteins, e.g. gp-120 and gp-41. Thus, approaches targeting gp-120 or gp-41 for the elimination of latently infected cells cannot work.

Other approaches to eliminate latent infected cells are based on eliminating the entire $CD4^+$ T-cell compartment (Berger (1998) Proc. Natl Acad. Sci. 95, 11511-11513), or the CD25-positive compartment (Bell (1993) Proc. Natl Acad. Sci. 90, 1411-1415) the CD45RO memory cell compartment (McCoig (1999) Proc. Natl. Acad. Sci 96, 11482-11485). However these markers do not adequately include all potentially infected cells.

Such cells include besides CD4-positive cells, or memory cells also macrophages, and non-hematopoietic cells.

In WO 98/18826 an antibody directed against the mammalian (e.g. human) chemokine receptor 5 is described and said antibody is proposed in a method of inhibiting the interaction of cell bearing CCR5 with a potential ligand, like HIV. It is proposed that said method inhibits an HIV infection. Furthermore, treatment options for inflammatory diseases, autoimmune diseases and graft rejection are proposed. Yet, all these treatment options are based on the assumption that specific antibodies like the immunoglobulin molecules themselves or functional portions thereof interfere with receptor-ligand interactions. However, whether these antibodies are capable of depleting the relevant cells is questionable. Furthermore, WO 98/18826 merely envisages the prevention of an interaction of HIV and the CCR5 receptor and thereby preventing an HIV infection.

Leukocytes, in particular T-cells, are believed to be the key regulators of the immune response to infective agents and are critical components for the initiation and maintenance of inflammatory processes, like inflammatory bowel disease inflammatory renal diseases, inflammatory joint disease, autoimmune disorders, like multiple sclerosis and arthritis, skin diseases, like psoriatic lesions, diabetes and in transplant rejection.

Therefore, the technical problem underlying the present invention is to provide for novel means and methods which can lead to the suppression of activated leukocytes involved in immunological pathologies, like autoimmune diseases, inflammation process and/or viral infections of immune cells.

Accordingly, the present invention relates to the use of an antibody and/or chemokine construct which binds to a chemokine receptor for the preparation of a pharmaceutical composition for the elimination of cells which are latently infected with a primate immunodeficiency virus, preferably a human immunodeficiency virus, most preferably HIV-1.

In context of the present invention the binding of said antibody and/or chemokine construct which binds to a chemokine receptor results in the depletion and/or destruction of

the target cell, namely the cell latently infected with said primate immunodeficiency virus.

In this invention it could surprisingly be shown that highly specific antibodies directed against an chemokine receptor were not able to destroy, lyse and/or deplete cells which express said chemokine receptor. However, antibody constructs or chemokine constructs as described and disclosed in the present invention were capable to specifically interact with said chemokine-receptor positive cells and were able to deplete said cells. Said depletion/destruction may, e.g., be achieved by the attraction of specific effector cells, like monocytes, macrophages, T-cells (particularly preferred are cytotoxic T-cells) or dendritic cells. Even if monoclonal antibodies had been shown to be successful in the destruction/depletion of malignant cells (see, e.g., Maloney (1999), Sem Oncol. 26, 76-78), they appear to be ineffective against certain subtypes of leukocytes, (comprising lymphocytes, polynuclear leukocytes and monocytes) especially $CCR5^+$ monocytes, T-cells and dendritic cells as documented herein and in the appended examples.

In accordance with the present invention, the term "antibody and/or chemokine construct" (i.e. antibody construct and/or chemokine construct) not only comprises the molecules and multifunctional constructs and compounds as described herein, but also comprises functional fragments thereof. Functional fragments of said constructs are meant to be fragments which are capable of binding to/interacting with a chemokine receptor on a target cell and providing for means for depleting, lysing and/or destroying said target cell.

Specific chemokine receptors, in accordance with the present invention comprise, but are not limited to, CXCR3, CXCR4, CXCR5, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, XCR1, CCR10 and CX3CR1. Chemokines and/or chemokine ligands binding to said chemokine receptors are well known in the art and shown, inter alia, in Table 4. Furthermore, chemokines and corresponding receptors are disclosed in Murphy (2000), Pharm. Reviews 52, 145-176. The chemokines, chemokine ligands and/or receptors are preferably primate, more preferably human chemokines/ligands/receptors.

The present invention also relates to the use of an antibody and/or chemokine construct which binds to a chemokine receptor for the preparation of a pharmaceutical

composition for the treatment, prevention and/or alleviation of inflammatory renal diseases, inflammatory bowel diseases, multiple sclerosis, skin diseases, allergic reactions diabetes or transplant rejection.

Said skin diseases comprise, inter alia, psoriatic disorders, atopic dermatitis or chronically inflamed skin. CCR6 expression is upregulated in PBMCs derived from patients with psoriasis. In addition, CCR6 ligand (CCL20=MIP3alpha) and CCR6 are upregulated in psoriatic skin. Furthermore, CCL20 expressing keratinocytes colocalize with skin infiltrating T-cells (Homey (2000) J. Immunol. 164, 6621-6632). Furthermore, CCR10 was detected on melanocytes, dermal fibroblasts, dermal endothelial cells, T-cells and skin-derived Langerhans cells but not keratinocytes. CCR10 ligand (CCL27) has a skin associated expression pattern (Homey (2000) J. Immunol. 164, 3465-3470; Charbonnier (1999) J. Exp. Med 190, 1755-1768). In addition, CCR4 and its ligand (TARC, MDC) are upregulated in chronically inflamed skin. Moreover CCR4 is a homing receptor for T-cells entering the skin. CCR4+ T-cells are only a small subpopulation of all T cells and therefore depletion of CCR4+ T-cells is indicated for various inflammatory skin diseases (Campbell (1999) Nature 400, 776-780). CCR3 and exotoxin expression is enhanced in atopic dermatitis and may contribute to the initiation and maintenance of inflammation (Yawalkar (1999) J. Invest. Dermatol. 113, 43-48).

Virtually all T-cells in rheumatoid arthritis, synovial fluid and in various inflamed tissues such as ulcerative colitis, chronic vaginitis and sarcoidosis express CXCR3. Whereas fewer T-cells within normal lymph nodes are CXCR3 positive.

For multiple sclerosis it was shown that CCR5 and CXCR3 are predominantly expressed on T-cells infiltrating demyelinating brain lesions, as well as in the peripheral blood of affected patients. The corresponding ligands MIP-1α and IP-10 were also detectable in the plaques (Balashov (1999) Proc. Natl. Acad. Sci. 96, 6873-6878). Elimination of the T-cells would block the T-cell arm of this autoimmune disease.

Immunochemical analysis of the expression of the beta-chemokine receptors in post-mortem CNS tissue from patients with multiple sclerosis revealed that in chronic active

MS lesions expression of CCR2, CCR3 and CCR5 was associated with foamy macrophages and activated microglia while low levels of these chemokine receptors were expressed by microglial cells in control CNS tissue. CCR2 and CCR5 were also present on large numbers of infiltrating lymphocytes and in 5/14 cases of MS CCR3 and CCR5 were also expressed on astrocytes. The elevated expression of CCR2, CCR3 and CCR5 in the CNS in MS suggests these beta-chemokine receptors and their ligands play a role in the pathogenesis of MS (Simpson, J. Neuroimmunol., 2000, 108, 192-200).

High expression of CCR3 and CCR5 was also observed in T cells and B cells of lymph nodes derived from patients with Hodgkin disease. While CCR3 was equally distributed in CD4+ and CD8+ cells, CCR5 was mainly associated with CD4+ cells. These data suggest that chemokines are involved in the formation of the nonneoplastic leukocytic infiltrates in Hodgkin disease (Buri, Blood, 2001, 97, 1543-8).

Periodontal disease is a peripheral infection involving species of gram-negative organisms. In patients with moderate to advanced periodontal disease CCR5 chemokine receptor expressing cells were found in the inflammatory infiltrates (Gamonal, J. Periodontal. Res., 2001, 36, 194-203 and Taubman, Crit. Rev. Oral. Biol. Med. 2001, 12, 125-35).

Diabetes type I is considered to be a T-cell mediated autoimmune disease. The expression of CCR5 receptor in the pancreas was associated with the progression of type I diabetes in relevant animal models (Cameron (2000) J. Immunol. 165, 1102-1110). In particular, the CCR5 expression was associated with the development of insulinitis and spontaneous type I diabetes.
Specific chemokines are associated with T-cell migration in diabetes type I relevant animal model: RANTES, MCP-1, MCP-3, MCP-5, IP10. These chemokines lead to a th1 immune response (Bradley (1999) J. Immunol. 162:2511-2520).

The above mentioned inflammatory bowel disease may comprise Morbus Crohn and colitis ulcerosa.

CCR9 is expressed on T-cells homing to the intestine and may be implied in Morbus Crohn and colitis ulcerosa. All intestinal lamina propria and intraepithelial lymphocytes express CCR9 (Zabel (1999) J. Exp. Med. 190, 1241-1256).

Additionally, the antibody- and/or chemokine construct as described in context of the present invention is also useful for avoiding complications during and/or after transplants, i.e. to avoid transplant rejections and graft versus host disease.
CCR7 is expressed on naïve T-cells and dendritic cells and mediates cell migration to lymphatic organs. Elimination of CCR7+ cells would therefore prevent an immune response to novel antigens, e.g., following transplantation. Such a treatment would not be generally immune suppressing but selective for novel antigens and limited for the duration of the administration of drugs of the invention depleting CCR7+ cells (Forster (1999) Cell 99, 23-33). CXCR5 is expressed on naïve B cells in the peripheral blood and tonsils and memory T-cells. Elimination of CXCR5+ B-cells would prevent the establishment of a humoral response. Furthermore, elimination of memory T-cells would reduce the cellular component of the immune response (Murphy (2000) Pharmacological Reviews 52, 145-176).

In order to provide pharmaceutical compositions for the treatment of allergies and/or allergic reactions, the antibody- and/or chemokine constructs as described herein may be employed. It was shown that CCR3 which binds exotoxin and RANTES, is expressed on eosinophils, Th2 cells, mast cells, basophils, which are involved in allergic reactions (Romangnani (1999) Am. J. Pathol. 155, 1195-1204).

As far as the above mentioned renal or kidney diseases are concerned, it has been shown that CCR5 positive T-cells may play a role in interstitial processes leading to fibrosis. CCR5 positive cells have been identified in the interstitial infiltrate of various glomevular and interstitial diseases, as well as transplant rejection. Said disease comprises acute and chronic nephritis, IgA nephropathy, and others (Segerer (1999), Kidney Int. 56, 52-64).

In a model of transient immune complex glomerulonephritis (IC-GN), CCR1, CCR2, and CCR5 were expressed early and were already downregulated at the peak of proteinuria and leukocyte infiltration. Expression of CCR5 was located to the glomerulus by in situ hybridization and quantitative reverse transcription-PCR of isolated glomeruli (Anders, J. Am. Soc. Nephrol., 2001, 12, 919-31). In kidneys of 38 patients with several renal diseases, CCR1- and CCR5-positive macrophages and T cells were detected in both glomeruli and interstitium as shown by immunohistochemistry. Renal CCR5-positive cells were dramatically decreased during convalescence induced by glucocorticoids (Furuichi, Am. J. Nephrol., 2000, 20, 291-9).

In a preferred embodiment of the present invention, the invention provides for the use of an antibody and/or chemokine construct which binds to a chemokine receptor for the preparation of a pharmaceutical composition as described hereinabove, wherein said chemokine receptor is the chemokine receptor 5 (CCR5). It is preferred that said CCR5 is the human CCR5.

The chemokine receptor CCR5 is a member of a large family of G protein coupled seven transmembrane domain receptors that binds the proinflammatory chemokines RANTES, MIP1-α, MIP1-ß and MCP-2. Chemokines act in concert with adhesion molecules to induce the extravasation of leukocytes and to direct their migration to sites of tissue injury.

The CCR5 is expressed on a minority of T-cells and monocytes and is further the major co-receptor for M-trophic HIV-1 strains that predominate early in the course of an HIV-infection.

The pharmaceutical composition as described hereinabove is, therefore, particularly useful in the depletion of $CCR5^+$ leukocytes and would be useful in the elimination of cells latently infected with HIV-1. Depletion of $CCR5^+$ cells should therefore reduce the number of cells latently infected with HIV and should be particularly useful in combination with active anti-viral, preferably anti-retroviral therapy.

In a particularly preferred embodiment said antibody construct is a bispecific antibody which binds to the chemokine receptor, preferably the CCR5, as a first antigen and a CD3 antigen of an effector cell as a second antigen. Preferably said CD3 antigen is on the surface of a T-cell, preferably a cytotoxic T-cell. Said CD3 therefore, denotes an antigen that is expressed on the above mentioned cells and may be part of the multi-molecular (T-) cell receptor complex.

Bispecific antibodies may be constructed by hybrid-hybridoma techniques, by covalently linking specific antibodies or by other approaches, like the diabody approach (Kipriyanow, Int. J. Cancer 77 (1998), 763-773).

It is preferred that said bispecific antibody is a single chain antibody construct.

As is well known, Fv, the minimum antibody fragment which contains a complete antigen recognition and binding site, consists of a dimer of one heavy and one light chain variable domain (V_H and V_L) in non-covalent association. In this configuration that corresponds to the one found in native antibodies the three complementarity determining regions (CDRs) of each variable domain interact to define an antigen binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen binding specificity to the antibody. Frameworks (FRs) flanking the CDRs have a tertiary structure which is essentially conserved in native immunoglobulins of species as diverse as human and mouse. These FRs serve to hold the CDRs in their appropriate orientation. The constant domains are not required for binding function, but may aid in stabilizing V_H-V_L interaction. Even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although usually at a lower affinity than an entire binding site (Painter, Biochem. 11 (1972), 1327-1337). Hence, said domain of the binding site of the antibody construct as defined and described in the present invention can be a pair of V_H-V_L, V_H-V_H or V_L-V_L domains of different immunoglobulins. The order of V_H and V_L domains within the polypeptide chain is not decisive for the present invention, the order of domains given hereinabove may be reversed usually without any loss of function. It is important, however, that the V_H and V_L domains are arranged so that the antigen binding site can properly fold.

Different parts of the antibodies/immunoglobulins can be joined by means of conventional methods or constructed as a contiguous protein by means of recombinant DNA techniques, e.g. in such a way that a nucleic acid molecule coding for a chimeric or humanized antibody chain is expressed in order to construct a contiguous protein (cf. for example Mack et al. (1995) Proc. Natl. Acad. Sci. USA, Vol. 92, pp. 7021-7025).

A single-chain antibody with the following Fv fragments is preferred: sc-Fv fragment of a monoclonal antibody against the chemokine receptor, preferably against CCR5, and an sc-Fv fragment of a monoclonal antibody against CD3. In this case, both the Fv fragment directed against the chemokine receptor and the Fv fragment against CD3 may be located in N-terminal position. The Fv fragment against CCR5 is preferred to be in N-terminal position. The order of the VL and VH antibody domains can be variable in both constructs, preferably, the order of the Fv fragment against CCR5 is VL-VH and the one of the Fv fragment against CD3 is VH-VL. The linkers between the variable domains as well between the two Fv fragments may consist of peptide linkers, preferably of a hydrophilic flexible glycine- and serine-containing linker of 1 to 25 amino acids. An additional histidine chain of ,e.g., 6 x His, in C- or N-terminal position can be used to simplify purification and detection.

Compared to conventional bispecific antibodies, bispecific single-chain antibodies have the advantage that they consist of only one protein chain and thus their composition is exactly defined. They have a low molecular weight of normally < 60 kD and can be produced easily and on a large scale in suitable cell lines, e.g. in CHO cells, using recombinant techniques. The most essential advantage, however, is that they have no constant antibody domains and thus only activate T-lymphocytes to lysis when these are bound to their target cells, i.e. to the chemokine-receptor expressing cells. Therefore, single-chain antibodies are often superior to conventional bispecific antibodies as their clinical use entails fewer or less severe side effects.

MC-1 was shown to bind specifically to the first part of the second extracellular loop of human CCR5 and did not crossreact with CCR5 derived from rhesus macaques as

shown in the appended examples. Therefore, it is preferred that said single chain antibody construct comprises V_L and V_H domains of a antibody specific for the chemokine receptor, preferably the human CCR5, and V_H and V_L domains of an antibody specific for a CD3 antigen. Said antibody specific for the human CCR5 is the murine anti-human CCR5 antibody MC-1, described, inter alia, in Mack (1998), J. Exp. Med. 187, 1215-1224 and in the appended examples. Yet, it is envisaged that other α-CCR5 antibodies, like MC-5 (as characterized in the appended examples and disclosed in Segerer (1999), loc. cit.) may be employed in the context of this invention. The antibody specific for a CD3 antigen may be selected from the group consisting of antibodies recognizing the gamma, delta, epsilon, zeta chains, particularly preferred are antibodies recognizing the epsilon chain and the CD3 zeta chain (Jakobs (1997) Cancer Immunol Immunother. 44, 257-264; Mezzanzanica (1991) Cancer Res 51, 5716-5721). Examples of anti-epsilon chain antibodies are OKT3 (WO 91/09968, Kung et al., Science 206, 347-349 (1979); Van Wauwe, J. Immunol. 124, 2708-2713 (1980); Transy, Eur. J. Immunol. 19, 947-950 (1989); Woodle, J. Immunol. 148, 2756-2763 (1992); Ada, Human. Antibod. Hybridomas, 41-47 (1994)) and TR66 (Traunecker (1991) EMBO J. 10, 3655-3659). Examples of monoclonal antibodies against the CD3 zeta chain are H2D9, TIA2 (both Becton Dickinson), G3 (Serotec Ltd.).

In a particularly preferred embodiment of the use of the present invention, the V_L and V_H domains of the single chain antibody as described above are arranged in the order V_L(MC-1)-V_H(MC-1)-V_H(CD3)-V_L(CD3), whereby it is particularly preferred that V_L(MC-1) comprises the amino acid sequence as depicted in SEQ ID NO: 12, wherein said V_H(MC-1) comprises the amino acid sequence as depicted in SEQ ID NO: 16, wherein said V_H(CD3) comprises the amino acid sequence as depicted in SEQ ID NO: 26 and/or wherein said V_L(CD3) comprises in SEQ ID NO: 28. Specific CDR parts of the MC-1 antibody are shown in SEQ ID NO: 29 to 34, wherein SEQ ID NO: 29 shows the CDR1 of V_L MC-1, SEQ ID NO: 30 shows the CDR2 of V_L MC-1, SEQ ID NO: 31 shows the CDR3 of V_L-MC-1, SEQ ID NO: 32 shows the CDR1 of V_H MC-1, SEQ ID NO: 33 shows the CDR2 of V_H MC-1 and SEQ ID NO: 34 depicts the CDR 3 of V_H MC-1.
Said bispecific antibody may, inter alia, comprise an amino acid sequence encoded by the nucleic acid sequence as depicted in SEQ ID NO: 17 or comprises the amino acid

sequence as depicted in SEQ ID NO: 18.

In another embodiment of the use of the present invention, the antibody construct is a bispecific antibody which binds to said chemokine receptor as a first antigen and a toxin as a second antigen. The antibody may be covalently bound to said toxin, and said antibody-toxin construct may be constructed by chemical coupling, producing a fusion protein or a mosaic protein from said antibody and from a modified or unmodified prokaryotic or eukaryotic toxin. Furthermore, said antibody may be joined to said toxin via additional multimerization domains.

In a further embodiment of the use of the present invention said antibody construct can, via a multimerization domain, be bound in vitro and/or in vivo to a second antibody construct which binds to a CD3 antigen and/or a toxin. Said multimerization may, inter alia, be obtained via hetero(di)merization. For example, the hetero(di)merization region of constant immunoglobulin domains may be employed. Other multi- and/or heterodimerization domains are known in the art and are based on leucine zippers, α- and ß-chains of T-cell receptors or MHC-class II molecules. Furthermore, jun- and fos-based domains may be employed (de Kuif (1996) J. Biol. Chem. 271, 7630-7634; Kostelny (1992), J. Immunol. 148, 1547-1553). Additional examples of multimerization domains are p53- and MNT-domains as described in Sakamoto (1994) Proc. Natl. Acad. Sci. USA 91, 8974-8978; Lee (1994) Nat. Struct. Biol. 1, 877-890; Jeffrey (1995) Science 267, 1498-5102 or Nooren (1999) Nat. Struct. Biol. 6, 755-759).

In another embodiment of the invention, the above mentioned chemokine construct is a fusion construct of a modified or an unmodified chemokine with a modified or an unmodified toxin. Said construct may, inter alia via a multimerization domain, be bound in vitro and/or in vivo to an antibody construct which binds to a CD3 antigen and/or to a toxin. Suitable multimerization domains have been described in the art and are mentioned hereinabove. The chemokine-toxin constructs may, inter alia, result from chemical coupling, may be recombinantly produced (as shown in the appended examples), or may be produced as a fusion protein from a chemokine and a modified or unmodified prokaryotic or eukaryotic toxin. It is particularly preferred that said chemokine binds to

the human chemokine receptor CCR5 and comprises, inter alia, RANTES, MIP-1α, MIP-1ß, MCP-2, MCP-3 or (a) fragment(s) thereof which are capable of binding to said receptor. A preferred toxin may be a truncated version of pseudomonas exotoxin, like PE38, PE40 or PE37. Most preferred, in context of this invention, is PE38.

Furthermore, and in accordance with the present invention, said chemokine construct may comprise the chemokine covalently bound to an antibody construct which binds to an antibody construct capable of binding to a CD3 antigen and/or which is a covalently bound to a toxin.

In a particularly preferred embodiment of the use of the present invention, the antibody and/or chemokine construct is a heterominibody construct comprising at least an antibody and/or a chemokine which binds to a chemokine receptor, preferably to the CCR5 receptor, most preferably to the human CCR5 receptor. Said heterominibody construct may comprise at least one toxin and it is particularly preferred that said heterominibody construct binds to the chemokine receptor as defined hereinabove and/or to a CD3 antigen of an effector cell. Preferred chemokines are the chemokines mentioned hereinabove and preferred toxins are the toxins described hereinabove, which may be modified or unmodified. Chemokines are well known in the art and described, inter alia, in Murphy (1999), loc. cit. Therefore, it is preferred that the chemokine is selected from the group consisting of RANTES, MIP-1ß, MIP-1α, MCP-2, and MCP-3 or a functional fragment thereof. The most preferred chemokine, in context of this invention is RANTES. Functional fragments of said chemokines are fragments which are capable of binding to or interacting with said chemokine receptor, preferably the human CCR5. Heterominibodies are known in the art and their production is, inter alia, described in WO 00/06605. Said heterominibody may be a multifunctional compound comprising at least one antibody and/or chemokine binding to or interacting with a chemokine receptor, preferably human CCR5, may (additionally) comprise a toxin as defined hereinbelow and/or a binding site for the CD3 antigen.

In a preferred embodiment, the antibody- or chemokine construct to be used in the present invention is a fusion (poly)peptide or a mosaic (poly)peptide. Said fusion

(poly)peptide may comprise merely the domains of the constructs as described herein above as well as (a) functional fragment(s) thereof. However, it is also envisaged that said fusion (poly)peptide comprises further domains and/or functional stretches. Therefore, said fusion (poly)peptide can comprise at least one further domain, said domain being linked by covalent or non-covalent bonds. The linkage as well as the construction of such constructs, can be based on genetic fusion according to the methods known in the art (Sambrook et al., loc. cit., Ausubel, "Current Protocols in Molecular Biology", Green Publishing Associates and Wiley Interscience, N.Y. (1989)) or can be performed by, e.g., chemical cross-linking as described in, e.g., WO 94/04686. The additional domain present in the construct may preferably be linked by a flexible linker, advantageously a (poly)peptide linker, wherein said (poly)peptide linker preferably comprises plural, hydrophilic, peptide-bonded amino acids of a length sufficient to span the distance between the C-terminal end of said further domain and the N-terminal end of the peptide, (poly)peptide or antibody or vice versa. Said linker may, inter alia, be a Glycine, a Serine and/or a Glycine/Serine linker. Additional linkers comprise oligomerization domains. Oligomerization domains facilitate the combination of two or several autoantigens or fragments thereof in one functional molecule. Non-limiting examples of oligomerization domains comprise leucine zippers (like jun-fos, GCN4, E/EBP; Kostelny, J. Immunol. 148 (1992), 1547-1553; Zeng, Proc. Natl. Acad. Sci. USA 94 (1997), 3673-3678, Williams, Genes Dev. 5 (1991), 1553-1563;Suter, "Phage Display of Peptides and Proteins", Chapter 11, (1996), Academic Press), antibody-derived oligomerization domains, like constant domains CH1 and CL (Mueller, FEBS Letters 422 (1998), 259-264) and/or tetramerization domains like GCN4-LI (Zerangue, Proc. Natl. Acad. Sci. USA 97 (2000), 3591-3595).

Furthermore, the antibody- or chemokine construct to be used in the present invention or as described hereinbelow may comprise at least one further domain, inter alia, domains which provide for purification means, like, e.g. histidine stretches. Said further domain(s) may be linked by covalent or non-covalent bonds.
The linkage can be based on genetic fusion according to the methods known in the art and described herein or can be performed by, e.g., chemical cross-linking as described in, e.g., WO 94/04686. The additional domain present in the construct as described and

disclosed in the invention may preferably be linked by a flexible linker, advantageously a polypeptide linker to one of the binding site domains wherein said polypeptide linker comprises plural, hydrophilic, peptide-bonded amino acids of a length sufficient to span the distance between the C-terminal end of one of said domains and the N-terminal end of the other of said domains when said polypeptide assumes a conformation suitable for binding when disposed in aqueous solution. Preferably, said polypeptide linker is a polypeptide linker as described in the embodiments hereinbefore. The polypeptide of the invention may further comprise a cleavable linker or cleavage site for proteinases, such as enterokinase

It is also envisaged that said constructs disclosed for uses, compositions and methods of the present invention comprises (a) further domain(s) which may function as immunomodulators. Said immunomodulators comprise, but are not limited to cytokines, lymphokines, T cell co-stimulatory ligands, etc.

Adequate activation resulting in priming of naive T-cells is critical to primary immunoresponses and depends on two signals derived from professional APCs (antigen-presenting cells) like dendritic cells. The first signal is antigen-specific and normally mediated by stimulation of the clonotypic T-cell antigen receptor that is induced by processed antigen presented in the context of MHC class-I or MHC class-II molecules. However, this primary stimulus is insufficient to induce priming responses of naive T-cells, and the second signal is required which is provided by an interaction of specific T-cell surface molecules binding to co-stimulatory ligand molecules on antigen presenting cells, further supporting the proliferation of primed T-cells. The term "T-cell co-stimulatory ligand" therefore denotes in the light of the present invention molecules, which are able to support priming of naive T-cells in combination with the primary stimulus and include, but are not limited to, members of the B7 family of proteins, including B7-1 (CD80) and 137-2 (CD86).

The antibody- and/or chemokine construct defined herein above or described hereinbelow may comprise further receptor or ligand function(s), and may comprise immunomodulating effector molecule or a fragment thereof. An immuno-modulating effector

molecule positively and/or negatively influences the humoral and/or cellular immune system, particularly its cellular and/or non-cellular components, its functions, and/or its interactions with other physiological systems. Said immuno-modulating effector molecule may be selected from the group consisting of cytokines, chemokines, macrophage migration inhibitory factor (MIF; as described, inter alia, in Bernhagen (1998), Mol Med 76(3-4); 151-61 or Metz (1997), Adv Immunol 66, 197-223), T-cell receptors and soluble MHC molecules. Such immuno-modulating effector molecules are well known in the art and are described, inter alia, in Paul, "Fundamental immunology", Raven Press, New York (1989). In particular, known cytokines and chemokines are described in Meager, "The Molecular Biology of Cytokines" (1998), John Wiley & Sons, Ltd., Chichester, West Sussex, England; (Bacon (1998). Cytokine Growth Factor Rev 9(2):167-73; Oppenheim (1997). Clin Cancer Res 12, 2682-6; Taub, (1994) Ther. Immunol. 1(4), 229-46 or Michiel, (1992). Semin Cancer Biol 3(1), 3-15).

Antibody and/or chemokine constructs as disclosed and described in the present invention and comprising (an) additional functional domain(s) may, inter alia, be multifunctional compounds, like heterominibodies, as described herein below.
The constructs to be used in the present invention or described herein may be constructs which comprise domains originating from one species, preferably from mammals, more preferably from human. However, chimeric and/or humanized constructs are also envisaged and within the scope of the present invention.

In a particular preferred embodiment, the composition of the invention comprises a constructs to be used in the present invention or described herein is a cross-linked (poly)peptide construct. As mentioned herein, said cross-linking may be based on methods known in the art which comprise recombinant as well as biochemical methods.

In a yet further embodiment of the use of the present invention, the antibody construct or the chemokine construct to be used comprises at least one toxin. Said toxin may be Pseudomonas exotoxin A, diphtheria toxin and similar toxins. It is envisaged that truncated toxins are employed, like the PE38 or the PE40 of Pseudomonas toxin described in the appended examples.

Said toxin may be bound to said antibody or chemokine by means as described hereinabove. It is also envisaged that said toxin is bound to the antibody/chemokine by means of a short peptide linker. The linker preferably consists of a flexible and hydrophilic amino acid sequence, in particular of glycines and serines. Preferably said linker has a length of 1 to 20 amino acids.

Several fusion proteins with a truncated version of Pseudomonas exotoxin A have been designed so far. Most of them have been used to target and destroy malignant cells. This toxin becomes activated upon proteolytic cleavage. A truncated version of the toxin (PE38) may be employed for the constructs of the present invention, as the full-length protein binds with its fist domain to the ubiquitous α2-macroglobulin receptor and is therefore toxic to most eukaryotic cells. Yet, this problem may be overcome by replacing the first domain of Pseudomonas exotoxin A by a specific sequence in order to alter the binding specificity of the toxin.

Furthermore, the present invention relates to the use of a chemokine construct which binds to a chemokine receptor for the preparation of a pharmaceutical composition for the elimination of cells which are latently infected with a primate immunodeficiency virus wherein said chemokine construct comprises a amino acid sequence as depicted in SEQ ID NO: 24 or as encoded by the nucleotide sequence as depicted in SEQ ID NO: 23.

As mentioned hereinabove, and in a preferred embodiment, the antibody and/or chemokine constructs to be used within the scope of the present invention bind to or interact with the CD3 antigen. Preferably said CD3 antigen is on the surface of an effector cell, namely a T-cell, preferably a cytotoxic T-cell.

It is particularly preferred that an antibody construct be used wherein said construct comprises a binding site for CCR5 and a binding site for CD3 and that a chemokine construct be used, wherein said chemokine construct comprises RANTES and said toxin is a truncated Pseudomonas exotoxin A (PE38).
The present invention, therefore also relates to antibody constructs comprising a bind-

ing site for CCR5 and a binding site for CD3 as well as to chemokine constructs comprising RANTES and the truncated Pseudomonas exotoxin A (PE38).

The present invention also relates to a polynucleotide encoding an antibody-construct as defined hereinabove or a polynucleotide encoding a chemokine construct as defined herein, wherein said polynucleotide is a polynucleotide comprising the nucleic acid molecule in particular encoding the polypeptide as depicted in SEQ ID NO: 18 or SEQ ID NO: 24; a polynucleotide comprising the nucleic acid molecule as depicted in SEQ ID NO: 17 or SEQ ID NO: 23; or (c) a polynucleotide hybridizing under stringent conditions to the complementary strand of a polynucleotide of (a) or (b).

With respect to the polynucleotides/nucleotide sequences characterized under (c) above, the term "hybridizing" in this context is understood as referring to conventional hybridization conditions, preferably such as hybridization in 50%formamide/6xSSC/0.1%SDS and 100µg/ml ssDNA, in which temperatures for hybridization are above 37°C and temperatures for washing in 0.1xSSC/0.1%SDS are above 55°C. Most preferably, the term "hybridizing" refers to stringent hybridization conditions, for example such as described in Sambrook., "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989. It is envisaged that the polynucleotides characterized under (c) above are highly homologous to the polynucleotides as defined in (a) and/or (b) and comprise a homology of at least 95%, more preferably of at least 97%, and most preferably 99% with the polynucleotides of (a) and/or (b).
Polynucleotides as defined and characterized under (c), therefore may encode for polypeptides being highly homologous to the polypeptides as defined in (a) and (b). The person skilled in the art can easily test the capacity of such homologous polypeptides to bind to chemokine receptors, in particular to the human CCR5 receptor and/or to eliminate, deplete and/or destroy cells, for example, cells which are infected by a primate immunodeficiency virus, like HIV-1, or eliminate, deplete and/or destroy target cells involved in immunological disorders or disclosed herein. The person skilled in the art can easily adopt the in vitro, in vivo and ex vivo experiments of the appended examples to verify the binding and/or depletion properties of such constructs.

Furthermore, said polynucleotide/nucleic acid molecule may contain, for example, thio-ester bonds and/or nucleotide analogues. Said modifications may be useful for the stabilization of the nucleic acid molecule against endo- and/or exonucleases in the cell. Said nucleic acid molecules may be transcribed by an appropriate vector containing a chimeric gene which allows for the transcription of said nucleic acid molecule in the cell. The polynucleotide/nucleic acid molecule of the composition of the present invention may be a recombinantly produced chimeric nucleic acid molecule comprising any of the aforementioned nucleic acid molecules either alone or in combination

Said polynucleotide may be, e.g., DNA, cDNA, RNA or synthetically produced DNA or RNA or a recombinantly produced chimeric nucleic acid molecule comprising any of those polynucleotides either alone or in combination. Preferably said polynucleotide is part of a vector. Such vectors may comprise further genes such as marker genes which allow for the selection of said vector in a suitable host cell and under suitable conditions. Preferably, the polynucleotide of the invention is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells. Expression of said polynucleotide comprises transcription of the polynucleotide into a translatable mRNA. Regulatory elements ensuring expression in eukaryotic cells, preferably mammalian cells, are well known to those skilled in the art. They usually comprise regulatory sequences ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers, and/or naturally-associated or heterologous promoter regions. Possible regulatory elements permitting expression in prokaryotic host cells comprise, e.g., the PL, lac, trp or tac promoter in E. coli, and examples for regulatory elements permitting expression in eukaryotic host cells are the AOX1 or GAL1 promoter in yeast or the CMV-, SV40-, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells. Beside elements which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as the SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. Furthermore, depending on the expression system used leader sequences capable of

directing the polypeptide to a cellular compartment or secreting it into the medium may be added to the coding sequence of the polynucleotide of the invention and are well known in the art; see also, e.g., the appended examples. The leader sequence(s) is (are) assembled in appropriate phase with translation, initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein, or a portion thereof, into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product; see supra. In this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (In-vitrogene), or pSPORT1 (GIBCO BRL).

Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming of transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and as desired, the collection and purification of the polypeptide of the invention may follow; see, e.g., the appended examples.

As described above, the polynucleotide of the invention can be used alone or as part of a vector to express the antibody- and/or chemokine constructs to be used in the invention or in cells, for, e.g., the treatment of immunological disorders or in anti-viral therapy. The polynucleotides or vectors containing the DNA sequence(s) encoding any one of the above described polypeptides is introduced into the cells which in turn produce the polypeptide of interest. Therefore, said polynucleotides and vectors may be used for gene therapy. Gene therapy, which is based on introducing therapeutic genes into cells by ex-vivo or in-vivo techniques is one of the most important applications of gene transfer. Suitable vectors, methods or gene-delivery systems for in-vitro or in-vivo gene therapy are described in the literature and are known to the person skilled in the art; see, e.g., Giordano, Nature Medicine 2 (1996), 534-539; Schaper, Circ. Res. 79 (1996), 911-919; Anderson, Science 256 (1992), 808-813; Verma, Nature 389 (1994), 239; Is-

ner, Lancet 348 (1996), 370-374; Muhlhauser, Circ. Res. 77 (1995), 1077-1086; Onodera, Blood 91 (1998), 30-36; Verma, Gene Ther. 5 (1998), 692-699; Nabel, Ann. N.Y. Acad. Sci. 811 (1997), 289-292; Verzeletti, Hum. Gene Ther. 9 (1998), 2243-51; Wang, Nature Medicine 2 (1996), 714-716; WO 94/29469; WO 97/00957, US 5,580,859; US 5,589,466; or Schaper, Current Opinion in Biotechnology 7 (1996), 635-640, and references cited therein. The polynucleotides and vectors of the invention may be designed for direct introduction or for introduction via liposomes, or viral vectors (e.g., adenoviral, retroviral) into the cell. Preferably, said cell is a germ line cell, embryonic cell, or egg cell or derived therefrom, most preferably said cell is a stem cell. An example for an embryonic stem cell can be, inter alia, a stem cell as described in, Nagy, Proc. Natl. Acad. Sci. USA 90 (1993), 8424-8428.

In accordance with the above, the present invention relates to vectors, particularly plasmids, cosmids, viruses and bacteriophages used conventionally in genetic engineering that comprise a polynucleotide encoding a polypeptide of the invention. Preferably, said vector is an expression vector and/or a gene transfer or targeting vector. Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of the polynucleotides or vector of the invention into targeted cell populations. Methods which are well known to those skilled in the art can be used to construct recombinant vectors; see, for example, the techniques described in Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory (1989) N.Y. and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1989). Alternatively, the polynucleotides and vectors of the invention can be reconstituted into liposomes for delivery to target cells. The vectors containing the polynucleotides of the invention can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts; see Sambrook, supra. Once expressed, the polypeptides of the present invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like; see, Scopes, "Protein Purification", Springer-Verlag, N.Y. (1982). Substantially pure polypeptides of at

least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity are most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically (including extracorporeally) or in developing and performing assay procedures.

In a still further embodiment, the present invention relates to a cell containing the polynucleotide or vector described above or to a host transformed with the vector of the invention. Preferably, said host/cell is a eukaryotic, most preferably a mammalian cell if therapeutic uses of the polypeptide are envisaged. Of course, yeast and less preferred prokaryotic, e.g., bacterial cells may serve as well, in particular if the produced polypeptide is used as a diagnostic means.

The polynucleotide or vector of the invention which is present in the host cell may either be integrated into the genome of the host cell or it may be maintained extrachromosomally.

The term "prokaryotic" is meant to include all bacteria which can be transformed or transfected with a DNA or RNA molecules for the expression of a polypeptide of the invention. Prokaryotic hosts may include gram negative as well as gram positive bacteria such as, for example, E. coli, S. typhimurium, Serratia marcescens and Bacillus subtilis. The term "eukaryotic" is meant to include yeast, higher plant, insect and preferably mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue. A polynucleotide coding for a polypeptide of the invention can be used to transform or transfect the host using any of the techniques commonly known to those of ordinary skill in the art. Especially preferred is the use of a plasmid or a virus containing the coding sequence of the polypeptide of the invention and genetically fused thereto an N-terminal FLAG-tag and/or C-terminal His-tag. Preferably, the length of said FLAG-tag is about 4 to 8 amino acids, most preferably 8 amino acids. Methods for preparing fused, operably linked genes and expressing them in, e.g., mammalian cells and bacteria are well-known in the art (Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989). The genetic constructs and methods described therein can be utilized for expression of

the polypeptide of the invention in eukaryotic or prokaryotic hosts. In general, expression vectors containing promoter sequences which facilitate the efficient transcription of the inserted polynucleotide are used in connection with the host. The expression vector typically contains an origin of replication, a promoter, and a terminator, as well as specific genes which are capable of providing phenotypic selection of the transformed cells. Furthermore, transgenic animals, preferably mammals, comprising cells of the invention may be used for the large scale production of the antibody- and/or chemokine construct of the invention. It is most preferred that said transgenic animal produces the antibody construct of the invention.

In a further embodiment, the present invention thus relates to a process for the preparation of a polypeptide described above comprising cultivating a (host) cell of the invention under conditions suitable for the expression of the antibody- and/or chemokine construct and isolating the polypeptide from the cell or the culture medium.
The transformed hosts can be grown in fermentors and cultured according to techniques known in the art to achieve optimal cell growth. The produced constructs of the invention can then be isolated from the growth medium, cellular lysates, or cellular membrane fractions. The isolation and purification of the, e.g., microbially expressed polypeptides of the invention may be by any conventional means such as, for example, preparative chromatographic separations and immunological separations such as those involving the use of monoclonal or polyclonal antibodies directed, e.g., against a tag of the polypeptide of the invention or as described in the appended examples.

Depending on the host cell, renaturation techniques may be required to attain proper conformation. If necessary, point substitutions seeking to optimize binding may be made in the DNA using conventional cassette mutagenesis or other protein engineering methodology such as is disclosed herein. Preparation of the polypeptides of the invention may also be dependent on knowledge of the amino acid sequence (or corresponding DNA or RNA sequence) of bioactive proteins such as enzymes, toxins, growth factors, cell differentiation factors, receptors, anti-metabolites, hormones or various cytokines or lymphokines. Such sequences are reported in the literature and available through computerized data banks.

The present invention further relates to an antibody construct or the chemokine construct encoded by the polynucleotide as described hereinabove or produced by the method described hereinabove.
Furthermore, the constructs of the invention can be used in the management of immunological disorders, in particular autoimmune diseases, allergic diseases, inflammatory diseases and AIDS (HIV-infection), as documented in the appended examples.

Additionally, the present invention provides for compositions comprising the polynucleotide, the vector, the host, the antibody construct and/or the chemokine construct of the invention.

The term "composition", in context of this invention, comprises at least one polynucleotide, vector, host, antibody construct and/or chemokine construct as described herein. Said composition, optionally, further comprises other molecules, either alone or in combination, like e.g. molecules which are capable of modulating and/or interfering with the immune system. The composition may be in solid, liquid or gaseous form and may be, inter alia, in a form of (a) powder(s), (a) tablet(s), (a) solution(s) or (an) aerosol(s). In a preferred embodiment, said composition comprises at least two, preferably three, more preferably four, most preferably compounds as described in the invention.

Preferably, said composition is a pharmaceutical composition further comprising, optionally, a pharmaceutically acceptable carrier, diluent and/or excipient.

Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions, etc. Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g., by intravenous, intraperitoneal, subcutaneous, intramuscular, topical or intradermal administration. Intravenous administration is particularly preferred. The dosage regiment will be determined

by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Generally, the regimen as a regular administration of the pharmaceutical composition should be in the range of 1 µg to 10 mg units per day. If the regimen is a continuous infusion, it should also be in the range of 1 µg to 10 mg units per kilogram of body weight per minute, respectively. However, a more preferred dosage for continuous infusion might be in the range of 0.01 µg to 10 mg units per kilogram of body weight per hour. Particularly preferred dosages are recited herein below. Progress can be monitored by periodic assessment. Dosages will vary but a preferred dosage for intravenous administration of DNA is from approximately 10^6 to 10^{12} copies of the DNA molecule. The compositions of the invention may be administered locally or systematically. Administration will generally be parenterally, e.g., intravenously; yet external administration is also envisaged. DNA may also be administered directed to the target site, e.g., by biolistic delivery to an internal or external target site or by catheter to a site in an artery. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishes, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. In addition, the pharmaceutical composition of the present invention might comprise proteinaceous carriers, like, e.g., serum albumin or immunoglobuline, preferably of human origin. Furthermore, it is envisaged that the pharmaceutical composition of the invention might comprise further biologically active agents, depending on the intended use of the pharmaceutical composition. Such agents might be drugs acting on the immunological system, drugs used in anti-viral treatment, in particular in HIV-treatment (for example,

HAART) and AIDS management and/or anti-inflammatory drugs. It is, for example, envisaged that patients are treated as early as possible with HAART until viral load is below detection level for several weeks to months. Early treatment of infected patients with HAART prevents the transition of viral strains from usage of CCR5 to other chemokine receptors, like CXCR4 (Connor (1997) J. Exp. Med. 185, 621-628). Constructs as disclosed in the present invention, for example, the CCR5xCD3 construct is administered in addition to HAART to eliminate latently infected cells as well as cells that are prone to reinfection by HIV-1. The depletion of $CCR5^+$ cells is repeated 1 to 10 times. Doses of CCR5xCD3 are in the range of 0.5μg/m2 to 10mg/m2, preferably 10μg/m2 to 100μg/m2. Doses can be administered intravenously, subcutaneously and/or into the cerebra-spinal fluid. After several treatment cycles with the bispecific antibody HAART is discontinued and viral load is closely monitored. If viral load increases above detection level, a new cycle of HAART and the bispecific antibody is initiated as described above.

It is envisaged by the present invention that the various polynucleotides and vectors of the invention are administered either alone or in any combination using standard vectors and/or gene delivery systems, and optionally together with a pharmaceutically acceptable carrier or excipient. Subsequent to administration, said polynucleotides or vectors may be stably integrated into the genome of the subject. Preferably said subject is a human.

On the other hand, viral vectors may be used which are specific for certain cells or tissues and persist in said cells. Suitable pharmaceutical carriers and excipients are well known in the art. The pharmaceutical compositions prepared according to the invention can be used for the prevention or treatment or delaying of different kinds of immunological diseases, which may be related to inflammation, in particular inflammatory bowel diseases, inflammatory renal diseases, inflammatory joint diseases like (chronic) arthritis. Furthermore, the pharmaceutical composition of the present invention may be employed to eliminate cells which are latently infected with a virus, preferably a primate immunodeficiency virus, more preferably with HIV(-1).

Furthermore, it is possible to use a pharmaceutical composition of the invention which comprises polynucleotide or vector of the invention in gene therapy. Suitable gene delivery systems may include liposomes, receptor-mediated delivery systems, naked DNA, and viral vectors such as herpes viruses, retroviruses, adenoviruses, and adeno-associated viruses, among others. Delivery of nucleic acids to a specific site in the body for gene therapy may also be accomplished using a biolistic delivery system, such as that described by Williams (Proc. Natl. Acad. Sci. USA 88 (1991), 2726-2729). Further methods for the delivery of nucleic acids comprise particle-mediated gene transfer as, e.g., described in Verma, Gene Ther.15 (1998), 692-699.

It is to be understood that the introduced polynucleotides and vectors express the gene product after introduction into said cell and preferably remain in this status during the lifetime of said cell. For example, cell lines which stably express the polynucleotide under the control of appropriate regulatory sequences may be engineered according to methods well known to those skilled in the art. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with the polynucleotide of the invention and a selectable marker, either on the same or separate plasmids. Following the introduction of foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows for the selection of cells having stably integrated the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines.

A number of selection systems may be used, including but not limited to, the herpes simplex virus thymidine kinase (Wigler, Cell 11 (1977), 223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska, Proc. Natl. Acad. Sci. USA 48 (1962), 2026), and adenine phosphoribosyltransferase (Lowy, Cell 22 (1980), 817) in tk^-, $hgprt^-$ or $aprt^-$ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, Proc. Natl. Acad. Sci. USA 77 (1980), 3567; O'Hare, Proc. Natl. Acad. Sci. USA 78 (1981), 1527), gpt, which confers resistance to mycophenolic acid (Mulligan, Proc. Natl. Acad. Sci. USA 78 (1981), 2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, J. Mol. Biol. 150 (1981), 1); hygro, which confers resistance to hygromycin (Santerre,

Gene 30 (1984), 147); or puromycin (pat, puromycin N-acetyl transferase). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, hisD, which allows cells to utilize histinol in place of histidine (Hartman, Proc. Natl. Acad. Sci. USA 85 (1988), 8047); and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McCologue, 1987, In: Current Communications in Molecular Biology, Cold Spring Harbor Laboratory ed.).

In a further embodiment, the present invention relates to a composition, preferably a pharmaceutical composition, as described hereinabove, which further comprises a medicament for the treatment of an immunological disorder or a medicament for anti-HIV treatment.
Said anti-HIV treatment may comprise HAART. HAART therapy consists of a cocktail of three classes anti-viral drugs. The classes are nucleosidal reverse transcriptase inhibitors (NRTI), non-nucleosidal reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PI). Usually 2 to 4 drugs from preferentially more than one class are combined to reduce viral load to almost non-detectable levels. Products, dosing schedules and common side effects are given in appended Tables I-III.
Said treatment of an immunological disorder may comprise anti-inflammatory agents and immunosuppressive agents. Anti-inflammatory agents may be selected from the group consisting of azathioprine, cyclophophamide, glucocorticoids like prednisone and corticosteroids. Immunosuppressive agents may comprise cyclosporin A, Tacrolimus (FK506), Sirolimus (Rapamycin). Protein drugs may comprise calcineurin, beta-interferon, anti-TNF alpha monoclonal antibodies (remicade). Dosing and use of anti-inflammatory agents and immunosuppressive agents is described, inter alia, in Fauci et al., sic. Further treatment options are known to the skilled artisan and, inter alia, described hereinabove.

In a particularly preferred embodiment, the present invention relates to a method for treating, preventing and/or alleviating an immunological disorder or for the elimination cells which are latently infected with a primate immunodeficiency virus comprising administering to a subject in need of such a treatment, prevention and/or alleviation an

effective amount of the compounds and/or compositions, preferably the pharmaceutical compositions of the present invention.

The constructs described herein are particularly useful in specifically destroying chemokine receptor positive cells. For example, a bispecific antibody, binding simultaneously to CCR5 on target cells and to CD3 on T-cells, redirects cytotoxic T-cells to the CCR5 positive target cells. As shown in the appended examples the antibody construct specifically depletes CCR5 positive T-cells and monocytes, but is inactive against cells that do not express CCR5 such as CCR5 deficient Δ32/Δ32 PBMC. Furthermore, in vitro/ex vivo experiments the bispecific antibody construct eliminated more than 95% of CCR5 positive monocytes and T-cells from the synovial fluid of patients with arthritis.
Other constructs, like chemokine constructs, for example, a fusion protein of the chemokine RANTES and a truncated version of the Pseudomonas exotoxin A (PE38) are able to bind to CCR5 and to downmodulate the receptor from the cell surface as exemplified in the appended examples. Within 48 h RANTES-PE38 completely destroyed CCR5 positive CHO cells at a concentration of 2 nM. No cytotoxic effect was detectable against CCR5 negative CHO cells.
Based on the predominance of CCR5 positive T-cells and monocytes in the infiltrate of chronically inflamed tissue, the specific depletion of CCR5 positive cells represents a new concept in the treatment of immunological disorders.

As described hereinabove, due to the fact that specific chemokine receptors are present on HIV-infected cells, namely CCR5, the compounds and compositions of the invention are particularly useful for the depletion/elimination of cells latently infected with primate immunodeficiency virus.

The present invention also relates to the use of the polynucleotide, the vector, the host, the antibody construct and/or the chemokine construct of the present invention for the preparation of a pharmaceutical composition for treating, preventing and/or alleviating an immunological disorder or for the preparation of a pharmaceutical composition for eliminating latently infected cells, wherein said cells are infected with a primate immunodeficiency virus, line a human immunodeficiency virus, in particular HIV-1.

Said immunological disorders may be autoimmune diseases, skin diseases, allergic diseases, inflammatory diseases, diabetes and transplant rejections, wherein said autoimmune disease is selected from the group consisting of multiple sclerosis, type I diabetes, rheumatoid arthritis. Said skin diseases, may comprise psoriatic lesions, psoriasis, atrophic dermatitis and the like. Inflammatory disease are mentioned hereinabove is selected from the group consisting of inflammatory joint diseases, inflammatory renal diseases, inflammatory bowel diseases. In particular, said inflammatory bowel disease may comprise Morbus Crohn, sarcoidosis, systemic sclerosis, collagenosis, myositis, neuritis. Inflammatory renal diseases may comprise nephritis, glomerulonephritis, lupus nephritis, or IgA nephropathy.

In a variety of chronic inflammatory diseases an impressive accumulation of CCR5 positive T-cells and macrophages is found at the site of inflammation. An accumulation of $CCR5^+$ cells has been demonstrated in several types of inflammatory diseases, like arthritis, inflammatory renal diseases, transplant rejection, auto-immune diseases like multiple sclerosis and inflammatory bowel diseases. In contrast, in the peripheral blood of these patients only a minority of T-cells and monocytes express CCR5. Therefore, CCR5 appears to be an excellent marker to identify leukocytes that are involved in chronic inflammation. The occurrence of a 32 bp deletion in the CCR5 gene which prevents expression of CCR5, allows to study the pathophysiological role of CCR5 in chronic inflammatory diseases. In patients with rheumatoid arthritis the frequency of CCR5 deficient ($\Delta32/\Delta32$) individuals is significantly reduced. Moreover, the mean survival of the kidney transplants is significantly longer in CCR5-$\Delta32/\Delta32$ patients. These results make CCR5 a target for therapeutic intervention. Furthermore, the prevalence of CCR5 positive leukocytes in the diseased tissue in contrast to the rare expression of CCR5 on the peripheral blood leukocytes means that a specific elimination of CCR5 positive leukocytes may be therapeutically useful by reducing the number of infiltrating cells in chronic inflammation, transplant rejection and autoimmune disease, like multiple sclerosis without significantly depleting peripheral blood leukocytes. Eliminating CCR5 positive leukocytes from the inflammatory infiltrate will be of greater therapeutic benefit than simply blocking chemokine receptors of these cells, as they have already infil-

trated the tissue.

As documented in the appended examples the antibody and/or chemokine constructs are particularly useful in the treatment, prevention and/or alleviation of inflammatory joint diseases. Therefore, the compositions of the present invention are particularly useful for the treatment of inflammatory joint diseases, like arthritis, in particular chronic arthritis.

The present invention furthermore, provides for medical methods and uses, wherein the composition, preferably the pharmaceutical composition, is to be administered in combination with antiviral agents and/or in combination with drugs to be employed in AIDS management.

As mentioned hereinabove, the main problem in AIDS management in the occurrence of latently HIV-infected cells. The current treatment options are based on anti-viral agents interfering with two enzymes of the HIV-1 virus, its protease and reverse transcriptase. The protease is essential to cleave the inactive viral pre-proteins to form the active products, while the reverse transcriptase is required to generate a DNA intermediate of the viral RNA genome. The DNA intermediate can then integrate into the host genome and remain there in a silent – latent form. The most efficient treatment option consists of highly active antiretroviral therapy (HAART) – a treatment regimen consisting of a combination of at least three anti-retroviral drugs and usually including at least one drug of the protease inhibitor class. The advent of highly active antiretroviral therapy (HAART) has had a significant impact on HIV-1-infected individuals, lowering circulating virus to undetectable levels (Oxenius (2000) Proc. Natl Acad. Sci. 97, 3383-3387; Perelson (1997) Nature (London) 387, 188-191; Hammer (1997) N. Engl. J. Med. 337, 725-733; Gulick (1997) N. Engl. J. Med. 337, 734-739). Despite this, latently infected cells can remain in these individuals for significant periods of time (Chun (1997) Nature (London) 387, 183-188; Chun (1998) Proc. Natl. Acad. Sci. USA 95, 8869-8873; Zhang (1999) N. Engl. J. Med. 340, 1605-1613); if HAART is withdrawn, these cells can produce virus (Harrigan (1999) AIDS 13, F59-F62). A pool of latently infected cells is generated early during primary HIV-1 infection (Chun (1998) Proc. Natl. Acd. Sci. 95,

8869-8873). Considering the postulated long half-life of latent viral reservoirs (Zhang (1999) N. Engl. J. Med. 340, 1605-1613, Finzi (1999) Nat. Med 5, 512-517) and the side effects and cost of chronic HAART (Flexner (1998) N. Engl. J. Med. 338, 1281-1292; Carr (1998) Lancet 351, 1881-1883), it is important to develop new strategies to eliminate the latent reservoir. While HAART treatment has been highly successful in suppressing plasma viremia in HIV-infected individuals, there are still persistent reservoirs of HIV including in latently infected CD4+ T-cells and other cells in the brain, gut associated lymphoid tissue and the genital tract (Chun (1999) Proc. Natl. Acad. Sci. 96, 10958-10961). Re-emergence of plasma viremia after discontinuation of HAART is due to those pre-existing viral reservoirs and HAART cannot eliminate those reservoirs (Chun (2000) Nature Med. 6, 757-761). Therefore, even HAART merely suppresses viral replication and reduces the viral load but does not prevent the occurrence of latent infected cells or eliminates such cells. Transmission of HIV-1 depends on the presence of CCR5, as individuals with a homozygous Δ32 deletion of the CCR5 allele are highly resistant against infection with HIV-1. Although highly active antiretroviral therapy can efficiently suppress replication of HIV-1, complete eradication of HIV has not been achieved to date. The main obstacle appears to be the inactivity of antiretroviral therapy against latently infected cells that can survive for several years and function as endogenous source for HIV-1. Many of these cells fail to express viral proteins and can evade the immune response. However, the majority of latently infected cells may still express CCR5, as this receptor is necessary for their initial infection. The compounds of the present invention are particularly useful in the depletion of $CCR5^+$ cells and should significantly reduce the number of latently infected HIV^+-cells. Other strategies to eliminate HIV-1 infected cells that depend on a specific recognition of viral proteins, e.g., surface expressed gp120, would be less effective against latently infected cells, as the virus is dormant in these cells.

Therefore, the compositions of the present invention are particularly useful in co-therapy approaches, which lead to a depletion of HIV-infected cells, preferably of CCR5 positive cells. It is preferred that the composition of the present invention is employed in combination with HAART. Therefore the construct of the invention may be used in HIV-therapy in combination with HAART as shown in the appended examples. Products,

dosing schedules and common side effects of HAART are known and illustrated, inter alia, in Tables I, II and III.
Said combination may comprise the co-administration as well as an administration before or after treatment with other anti-viral, preferably anti-retroviral, most preferably anti-HIV medication.

The present invention also provides for a kit comprising the polynucleotide, the vector, the host, the antibody construct and/or the chemokine construct of the present invention.

Advantageously, the kit of the present invention further comprises, optionally (a) storage solution(s) and/or remaining reagents or materials required for the conduct of scientific or therapeutic methods. Said kit may, inter alia, comprise drugs and/or medicaments employed in the treatment of immunological disorders as defined herein and/or in AIDS management. Furthermore, parts of the kit of the invention can be packaged individually in vials or bottles or in combination in containers or multicontainer units.

The Figures show:

Figure 1.

Expression of various chemokine receptors (indicated on the x-axis) on T cells (first and second panel), monocytes (third panel) and neutrophils (forth panel) in the peripheral blood (white columns) and the synovial fluid (gray columns) of patients with arthritis other than gout. Each dot represents one patient and mean values are given as bars. Expression of CXCR1 and CXCR2 on neutrophils is given as fluorescence intensity on the y-axis, while in all other cases the percentage of receptor positive cells is depicted.

Figure 2.

FACS dot plots showing the expression of CCR5, CCR2 and CXCR4 on leukocytes in the peripheral blood (left) and synovial fluid (right) of one patient with rheumatoid arthritis. The cut-offs were set according to the isotype controls and are shown as vertical lines. In the synovial fluid the majority of T-cells and monocytes show a high level of

CCR5 expression, while in the peripheral blood only a minority of these cells express low levels of CCR5.

Figure 3.

Scheme of the bispecific single-chain antibody. The αCCR5 single-chain fragment (CCR5 VL / CCR5 VH) derived from the hybridoma MC-1 is fused to the N-terminus of a single-chain fragment directed against CD3 (CD3 VH / CD3 VL). Binding of the bispecific antibody to CD3+ T cells and CCR5 positive target cells results in crosslinkage of CD3, activation of effector T cells and lysis of CCR5 positive target cells.

Figure 4.

SDS page of the purified bispecific single-chain antibody αCCR5-αCD3. A single band of approx. 60 kD is visible under reducing (left) and non-reducing (right) conditions. No degradation or proteolysis of the bispecific antibody is detectable.

Figure 5.

Scheme of the chemokine-toxin RANTES-PE38. The chemokine RANTES is fused to the N-terminus of a truncated version of the Pseudomonas exotoxin A (PE38). While the truncated toxin is unable to bind to eukaryotic cells, the fusion protein binds with the RANTES moiety to CCR5 and becomes internalized into the cell. Thereby the toxin inhibits protein synthesis and induces cell death.

Figure 6.

SDS-PAGE (left) and Westernblot (right) of the purified protein RANTES-PE38. A distinct band with the expected size of approx. 46 kD is visible in the coomassie stained SDS-PAGE and Westernblot.

Figure 7.

Binding of the αCCR5-αCD3 bispecific antibody to CD3 on CCR5 deficient lymphocytes. Costaining with CD4 and CD8 demonstrated that the bispecific antibody binds to the subpopulation of CD4+ / CD8+ T cells. Multicolor analysis showed that no binding to other cell populations occurred.

Figure 8.

Binding of the αCCR5-αCD3 bispecific antibody to CCR5 on transfected CHO cells. CHO cells transfected with CCR5 are shown in black, while CXCR4 positive CHO cells served as negative control and are shown in white.

Figure 9.

Binding of the αCCR5-αCD3 bispecific antibody to CCR5 on cultured monocytes. Monocytes from a CCR5 positive donor are shown in black, while monocytes from a CCR5 deficient (Δ32/Δ32) donor served as negative control and are shown in white.

Figure 10.

CCR5 specific monoclonal antibodies were compared in their ability to induce down-modulation of CCR5 as analyzed by FACS. MAb MC-1 (squares), the parental antibody of the αCCR5-αCD3 bispecific antibody, showed significant internalization, while MC-4 (triangle) showed no induction of CCR5 internalization. CHO-CCR5 cells were incubated with various concentrations for 30 min at 37°C.

Figure 11.

Downmodulation of CCR5 from the surface of PBMC with RANTES-PE38 (open symbols) and RANTES (closed symbols). Surface expression of CCR5 was determined on lymphocytes (squares) and monocytes (circles) and is given as % of the medium control. The fusion protein RANTES-PE38 is able to downmodulate CCR5 from the cell surface with a somewhat lower efficiency than unmodified RANTES.

Figure 12.

Depletion of CCR5 positive monocytes by the bispecific antibody. CCR5 deficient PBMC (Δ32/Δ32) or wildtype PBMC (WT-PBMC) were cultured overnight and incubated with the bispecific antibody (100 ng/ml) or medium as control for 20 h. Remaining monocytes (Mo) and lymphocytes (Ly) were identified by their light scatter properties in FACS. The CCR5 positive wildtype monocytes were completely depleted by the bispecific antibody, while the CCR5 deficient monocytes survived.

Figure 13.

Depletion of CCR5 positive monocytes by the bispecific antibody. Dose response showing depletion of cultured monocytes with various concentrations of the αCCR5-αCD3 bispecific antibody. More than 90 % of monocytes were depleted at a concentration of 33 ng/ml.

Figure 14.

The bispecific αCCR5-αCD3 antibody depletes lymphocytes and monocytes from the synovial fluid of a patient with chronic arthritis. Freshly draw synovial fluid was incubated with various concentrations of the bispecific antibody or medium as control for 20 h and analyzed by FACS. More than 95 % of both cell types were depleted at a concentration of 31 ng/ml.

Figure 15.

The bispecific αCCR5-αCD3 antibody depletes lymphocytes and monocytes from the synovial fluid of a patient with chronic arthritis. Freshly draw synovial fluid was incubated with the bispecific antibody (500 ng/ml) or medium as control for 20 h and analyzed by FACS (forward and sideward light scatter analysis). The bispecific antibody completely depleted the CCR5 positive monocytes and lymphocytes, while the CCR5 negative granulocytes (PMN) survived. Consistent with our previous data all monocytes and lymphocytes in this synovial fluid expressed CCR5, while no expression of CCR5 was found on granulocytes (PMN).

Figure 16.

The efficacy of the αCCR5-αCD3 bispecific single-chain antibody in depleting CCR5 positive monocytes was compared with the efficacy of two unmodified monoclonal antibodies MC-1 and MC-5. PBMC from two different donors (F and N) were cultured overnight and then incubated for 24 h with medium in the presence or absence of antibody construct and antibody. Concentrations were as indicated. The cells were completely recovered and analyzed by FACS to quantify surviving monocytes and lymphocytes. Shown are the results of two experiments per PBMC donor. Surprisingly only the

bispecific antibody was able to considerably deplete CCR5 positive monocytes, while the unmodified monoclonal antibodies were largely ineffective.

Figure 17.

Example of the forward and sideward light scatter analysis of a representative experiment as shown in Fig. 16, indicating that only the αCCR5-αCD3 bispecific single-chain antibody was capable of depleting the monocytes in the left lower quadrants. For comparison of the localization of different cell types also see Figure 12 left panel.

Figure 18.

Destruction of CCR5 positive CHO cells with the chemokine-toxin RANTES-PE38. CCR5 positive CHO cells and CXCR4 positive CHO cells were incubated for 40 h with the chemokine-toxin (10 nM) and analyzed by FACS. Dead cells appear in the left upper region of the forward and sideward light scatter plot. RANTES-PE38 completely destroyed the CCR5 positive CHO cells while it had no effect on the CXCR4 positive CHO cells.

Figure 19.

Examples of antibody and / or chemokine constructs binding to chemokine receptor (CCR) expressing cells that are combined by peptide linkage or by multimerization domains: (A) shows various examples of antibody and chemokine constructs that interact with an effector cell by binding to an effector cell surface antigen, (B) shows examples of antibody and chemokine constructs that are linked to a toxin, (C) shows examples of antibody and chemokine constructs, that contain an antibody binding site for a toxin.

Figure 20.

$4x10^5$ CCR5+CHO cells were incubated with 19,5 ng/ml scFV CCR5xCD3 for 30 minutes at 4°Cafter washing cells were incubated for 45 minutes at 4°C with 20 µg/ml anti-His-Tag monoclonal antibody. Binding of scFV CCR5xCD3 was detected with a monoclonal goat anti-mouse IgG $F(ab')_2$-PE conjugated antibody and analysed in a flow cytometer using the CellQuest software. Nonlinear regression analysis was performed with GraphPad Prizm.

Figure 21.

The cytotoxic activity of scFv CCR5xCD3 was tested in a FACS based assay with CCR5+CHO as target and CD3+ T-lymphocytes as effector cells. CD3+ T-cells were isolated from peripheral blood. CCR5+CHO cells were labeled with 12 µM PKH26. Effector:target cells in a ratio of 5:1 were incubated with dilutions of scFv CCR5xCD3 ranging from 320 ng/ml to 0.3 pg/ml for 16 hours at 37°C and 5% CO_2. After staining with 1 µg/ml propidium iodine (PI), cells were analyzed by flow cytometry.

In order to verify the specificity of scFv CCR5xCD3 mediated lysis, stably CXCR4 transfected CHO cells were used as negative control target cells. The cytotoxicity assay was performed under identical conditions as described for CCR5+CHO cells.

Specific lysis of CCR5+CHO cells was calculated using the CellQuest software (Becton Dickinson) and nonlinear regression analysis was performed with GraphPad Prizm. The sigmoidal dose response curve revealed an EC50 value of 912 pg/ml. No cytotoxic effect of scFv CCR5xCD3 was observed using CXCR4+ CHO cells as target cells.

Figure 22.

The cytotoxic activity of scFv CCR5xCD3 on CCR5-positive cells was also tested using the CD3 positive T-cell line CB15 as effector cells in a FACS based assay. CCR5+CHO target cells labeled with 10 µM PKH26 were used in a effector:target ratio of 10:1 and incubated with 100 µl of scFv CCR5xCD3 in different dilutions (40 µg/ml to 0.15 ng/ml) for 6 hours at 37°C at 5% CO_2. Cells were stained with 1 µg/ml propidium iodine (PI) and analyzed in duplicate in a flow cytometer.

Specific lysis of CCR5+CHO cells was calculated using the CellQuest software (Becton Dickinson) and nonlinear regression analysis was performed with GraphPad Prizm. The sigmoidal dose response curve revealed an EC50 value of 12.8 ng/ml.

Figure 23.

Reactivity of MC-1 with A) human PBMC and B) rhesus PBMC. PBMC (solid line), PBMC with PE conjugated goat anti-mouse antibody (dotted line) and PBMC with MC-1 and PE conjugated goat anti-mouse antibody (solid bold line). Binding of MC-1 to human PBMC (A), but not to rhesus PBMC (B) is indicated by the M1 marker line.

The invention will now be described by reference to the following biological examples which are merely illustrative and are not to be construed as a limitation of scope of the present invention.

Example 1: Cell lines, PBMC preparation, synovial fluid

1.1 Generation of a CHO cell line expressing human CCR5

The cDNA sequence of CCR5 was amplified from genomic DNA of human PBMC by PCR with Pfu-polymerase (Stratagene), oligonucleotide primers were:

SEQ ID NO. 1 : 5′ GGA ACA AGA TGG ATT ATC AAG TGT C 3′

SEQ ID NO. 2: 5′ CTG TGT ATG AAA ACT AAG CCA TGT G 3′

The amplified fragment was gel purified, ligated into the PCR-Script Amp Sk(+) script vector (Stratagene) and sequenced. After subcloning into the PEF-DHFR vector, DHFR-deficient CHO cells were transfected by electroporation and selected for stable expression in nucleoside free MEM medium with 10% dialyzed FCS as described. The CHO/CCR5 transfected cells were shown to be homogeneous by FACS-analysis.

1.2 PBMC purification

PBMC were isolated from buffy coats or full blood of healthy donors by ficoll density gradient centrifugation. Where indicated PBMC were used from donors with a homozygous 32 basepair deletion in the CCR5 allele (Δ32/Δ32) preventing surface expression of CCR5. Specifically, buffy coats were diluted 1:2 in 0.9% NaCl, and 35 ml were layered onto 15 ml of Ficoll Paque and centrifuged for 25 min at 400 g. The white interphase was harvested and thrombocytes depleted by three subsequent washing and centrifugation steps at 100 g for 6 min in RPMI with 10% FCS. Freshly isolated monocytes expressed a very low level of CCR5, but expression was strongly induced after culture of PBMC in RPMI with 10% FCS for 24 to 48 h at 37°C. The amount of FCS did not influence this induction. The expression of CCR5 on lymphocytes was not altered during culture.

1.3 Synovial fluid

Synovial fluid of patients with arthritis was obtained from diagnostic or therapeutic arthrocentesis and used for the experiments without further preparation. Informed consent was obtained from all patients. Synovial fluid and blood samples were simultaneously obtained from 23 patients who presented with gonarthritis for diagnostic or therapeutic arthrocentesis. Diagnoses included rheumatoid arthritis (7), reactive arthritis (3), undifferentiated gonarthritis (4), psoriatic arthritis (3), osteoarthritis (2), ancylosing spondylitis (1) and gout (3) according to ACR criteria, where applicable. Written informed consent was obtained from all patients. Synovial fluid was analyzed by light microscopy. Crystals were identified by polarized light microscopy. Student's t-test and paired t-test was applied for statistical analysis.

1.4 Analysis of chemokine receptor expression in whole blood samples and synovial fluid

Immediately after arthrocentesis SF (synovial fluid) leukocytes were isolated by two washing steps with 5% PBS in NaCl 0.9%. Synovial fluid cells and whole blood (containing 1 mM EDTA) were incubated on ice with monoclonal antibodies against chemokine receptors and the appropriate isotype controls at a concentration of 10µg/ml. The antibodies were for CCR5: MC-1 (Mack (1998) J. Exp. Med. 187, 1215-1224), for CCR2: DOC-3, which specifically binds to CCR2 (9), for CCR1: Clone 53504 (R&D-Systems), for CXCR1: 5A12 (Pharmingen), for CXCR2: 6C6 (Pharmingen), and for CXCR4 12G5 (Pharmingen), IgG1-, IgG2a- and IgG2b-isotype controls (Sigma). After two washing steps cells were incubated for 30 min on ice with a PE-conjugated rabbit-anti-mouse F(ab)2 fragment (R439, DAKO). Cells were washed twice and incubated with 10% mouse serum followed by a combination of CD4-FITC, CD8-PECy5 and CD14-APC (Immunotech). After lysis of erythrocytes, cells were immediately analyzed by flow cytometry (Becton-Dickinson). Calculations were performed with Cell Quest analysis software. Helper T cells, cytotoxic T cells, monocytes and neutrophils were identified by their light scatter properties and the expression or absence of CD4, CD8 and CD14. Chemokine receptor expression was calculated after defining a cutoff according to the isotype control.

In both acute and chronic joint effusions we found a consistently increased percentage of CD4+ and CD8+ T cells that expressed the chemokine receptor CCR5 compared to the peripheral blood. These data are in good agreement with previous reports (Mack (1999) Arthritis Rheum. 42, 981-988; Qin (1998) J. Clin. Invest. 101, 746-754).

Chemokine receptor expression on T cells in non-crystal induced arthritis: Approximately 88% of CD4+ T cells and 93% of the CD8+ T cells from the synovial fluid stained positive for the chemokine receptor CCR5. Similarly, a major proportion of CD8+ and CD4+ T cells in the SF expressed CCR2 (66% and 48%) (Fig. 1). In contrast, in the peripheral blood only a minority of T cells expressed the chemokine receptors CCR5 or CCR2. The enrichment in the synovial fluid was most pronounced for the CCR5+ helper-T cells (blood: SF ratio = 1:4). The majority of T lymphocytes stained positive for CXCR4 in both compartments. CXCR1, CXCR2 and CCR1 were only expressed by a minor and variable percentage of T cells (Fig. 1). A typical example of one patient is shown in Fig. 2, showing the expression of CCR5, CCR2 and CXCR4 on leukocytes in the peripheral blood and synovial fluid.

Chemokine receptor expression on monocytes in non-crystal induced arthritis: Consistent with previous data, the majority of monocytes in the SF expressed CCR5. In addition, a reduced expression of CXCR1, CXCR2, CXCR4 and CCR1 is here reported on monocytes in the synovial fluid compared to the peripheral blood (Fig. 1, 2). Not only was a lower frequency of receptor positive cells found, but also a lower density of chemokine receptors on the cell surface (data not shown). No differences could be detected in relation to the underlying diagnoses, duration of joint effusion or pretreatment. CCR2 was equally expressed by all monocytes in both compartments (Fig.'s 1, 2).

Chemokine receptor expression on neutrophils in non-crystal induced arthritis: Acute arthritis is characterized by a rapid influx of neutrophils into the inflamed joint. Therefore, the chemokine receptor expression on neutrophils from inflamed joint effusions was analyzed. For the first time a high expression of CXCR4 is described on a large fraction of neutrophils (60%) from the synovial fluid of patients with acute and chronic arthritis, while a much lower expression was found in the peripheral blood (24 %) (Fig. 1, 2). In arthritis other than gout CXCR1 and CXCR2 was reduced on neutrophils from the synovial fluid by approximately 50% compared to the peripheral blood. CCR1 was expressed only by a minority of neutrophils in both compartments.

1.5 Determination of CCR5 genotype

Genomic DNA was prepared from frozen blood samples by affinity chromatography (Roche Diagnostics). Subsequently a fragment of the CCR5 gene containing the potential 32 basepair deletion was amplified by a 40 cycle PCR with Taq polymerase. The primers were

SEQ ID NO. 3: 5′ TTT ACC AGA TCT CAA AAA GAA G 3′

SEQ ID NO. 4: 5′ GGA GAA GGA CAA TGT TGT AGG 3′

Differences in the length of the PCR fragments (274 or 242 bp) allowed to identify CCR5-wildtype and CCR5-Δ32 alleles.

Example 2: Construction of a bispecific antibody

2.1 Generation of monoclonal antibodies against human CCR5

To generate monoclonal antibodies against human CCR5, five BALB/c mice were immunized intraperitoneally (i.p.) at four week intervals, first with $1x10^7$ PBMC cultured for 10 days in IL-2 (100 U/ml) and six subsequent i.p. injections of $1x10^7$ CHO cells expressing high levels of CCR5. For this purpose, CCR5 transfected CHO cells were grown in the presence of 20 nM methotrexate to amplify expression of CCR5 and one clone expressing high levels of CCR5 was chosen. Four days after the last i.p. injection of CHO/CCR5 cells, the spleens were removed and the cells fused with the P3X63-Ag8 cell line. Supernatants from approximately 6000 hybridomas were screened per fusion by flow cytometry on stable CHO/CCR5 cells and monoclonal antibodies against CCR5 (MC-1, MC-4, MC-5) were detected after the third fusion. The specificity of MC-1 (IgG1), MC-4, MC-5 were tested on CHO cells stably transfected with CCR1-3 and CXCR4. In all cases no binding was detected. In addition the antibodies did not react with freshly isolated PBMCs and cultured PBMCs from a donor homozygous for the Δ32 deletion in the CCR5 gene.

2.2 Cloning of the variable domains of MAb MC-1 against CCR5

The light (VL) and heavy (VH) variable domains from the αCCR5 hybridoma MC-1 were cloned using PCR amplification (Orlandi (1989) Proc. Natl. Acad. Sci. 86, 3833).

Reverse transcription was carried out with random hexamer nucleotides and Super-Script reverse transcriptase (Gibco). The variable domains were amplified by PCR with Pfu-polymerase, subcloned into the vector PCR-script Amp SK+ (Stratagene) and sequenced.

For PCR amplification of VL(1) the following primers were used:

SEQ ID NO. 5: 5′ GACATTCAGC TGACCCAGTC TCCA 3′

SEQ ID NO. 6: 5′ GTTTTATTTC CAGCTTGGTC CC 3′

For PCR amplification of VH(1) the following primers were used:

SEQ ID NO. 7: 5′ ACCATGGGAT GGAGCTGTGT CATGCTCTT

SEQ ID NO. 8: 5′ TGAGGAGACG GTGACCGTGG TCCCTTGGCC CCAG

The nucleotide sequence of VL(1) obtained by RT PCR is SEQ ID NO. 9:

```
  1 GACATTCAGC TGACCCAGTC TCCAGCCTCC CTATCTGCAT CTGTGGGAGA AACTGTCACC
 61 ATCACATGTC GAGCAAGTGA GAATATTTAC AGTTATTTAG CATGGTATCA GCAGAAACAG
121 GGAAAATCTC CTCAACTCCT GGTCTATAAT GCAAAAACCT TAACAGAAGG TGTGCCATCA
181 AGGTTCAGTG GCAGTGGATC AGGCACACAG TTTTCTCTGA AGATCAACAG CCTGCAGCCT
241 GAAGATTTTG GGAATTATTT CTGTCAACAT CATTATGATA CTCCTCGGAC GTTCGGTGGA
301 GGGACCAAGC TGGAAATAAA AC
```

The corresponding translated protein sequence to VL(1) is SEQ ID NO. 10:

```
 1 D I Q L T Q S P A S L S A S V G E T V T I T C R A S E N I Y
31 S Y L A W Y Q Q K Q G K S P Q L L V Y N A K T L T E G V P S
61 R F S G S G S G T Q F S L K I N S L Q P E D F G N Y F C Q H
91 H Y D T P R T F G G G T K L E I K
```

The nucleotide sequence of VL(1) without the primer sequences used for amplification, SEQ ID NO. 11:

```
  1 GCCTCCCTAT CTGCATCTGT GGGAGAAACT GTCACCATCA CATGTCGAGC AAGTGAGAAT
 61 ATTTACAGTT ATTTAGCATG GTATCAGCAG AAACAGGGAA AATCTCCTCA ACTCCTGGTC
121 TATAATGCAA AAACCTTAAC AGAAGGTGTG CCATCAAGGT TCAGTGGCAG TGGATCAGGC
181 ACACAGTTTT CTCTGAAGAT CAACAGCCTG CAGCCTGAAG ATTTTGGGAA TTATTTCTGT
241 CAACATCATT ATGATACTCC TCGGACGTTC GGTGGA
```

The corresponding translated protein sequence to SEQ ID NO. 11: of VL(1) is SEQ ID NO. 12:

```
 1 A S L S A S V G E T V T I T C R A S E N I Y S Y L A W Y Q Q
31 K Q G K S P Q L L V Y N A K T L T E G V P S R F S G S G S G
61 T Q F S L K I N S L Q P E D F G N Y F C Q H H Y D T P R T F
```

```
91 G G
```

The sequence of VH(1) including the leader sequence obtained by RT PCR is SEQ ID NO. 13:

```
  1 ATGGGATGGA GCTGTGTCAT GCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
 61 GTCCAACTGC AGCAGCCTGG GGCTGGGAGG GTGAGGCCTG GAGCTTCAGT GAAGCTGTCC
121 TGCAAGGCTT CTGGCTACTC CTTCACCAGT TACTGGATGA ACTGGGTGAA GCAGAGGCCT
181 GGACAAGGCC TTGAGTGGAT TGGCATGATT CATCCTTCCG ATAGTGAAAC TAGGTTAAAT
241 CAGAAGTTCA ACGACAGGGC CACATTGACT GTTGACAAAT ATTCCAGCAC AGCCTATATA
301 CAACTCAGCA GCCCGACATC TGAGGACTCT GCGGTCTATT ACTGTGCAAG AGGAGAATAT
361 TACTACGGTA TATTTGACTA CTGGGGCCAA GGGACCACGG TCACCGTCTC CTCA
```

The corresponding translated protein sequence to VH(1) is SEQ ID NO. 14 :

```
  1 M G W S C V M L F L V A T A T G V H S Q V Q L Q Q P G A G R
 31 V R P G A S V K L S C K A S G Y S F T S Y W M N W V K Q R P
 61 G Q G L E W I G M I H P S D S E T R L N Q K F N D R A T L T
 91 V D K Y S S T A Y I Q L S S P T S E D S A V Y Y C A R G E Y
121 Y Y G I F D Y W G Q G T T V T V S S
```

The nucleotide sequence of VH(1) without the leader sequence and primer sequences used for amplification, SEQ ID NO. 15:

```
  1 CTTGGTAGCA ACAGCTACAG GTGTCCACTC CCAGGTCCAA CTGCAGCAGC CTGGGGCTGG
 61 GAGGGTGAGG CCTGGAGCTT CAGTGAAGCT GTCCTGCAAG GCTTCTGGCT ACTCCTTCAC
121 CAGTTACTGG ATGAACTGGG TGAAGCAGAG GCCTGGACAA GGCCTTGAGT GGATTGGCAT
181 GATTCATCCT TCCGATAGTG AAACTAGGTT AAATCAGAAG TTCAACGACA GGGCCACATT
241 GACTGTTGAC AAATATTCCA GCACAGCCTA TATACAACTC AGCAGCCCGA CATCTGAGGA
301 CTCTGCGGTC TATTACTGTG CAAGAGGAGA ATATTACTAC GGTATATTTG ACTA
```

The corresponding translated protein sequence to SEQ ID NO. 15 of VH(1) is SEQ ID NO. 16:

```
 1 L V A T A T G V H S Q V Q L Q Q P G A G R V R P G A S V K L
31 S C K A S G Y S F T S Y W M N W V K Q R P G Q G L E W I G M
61 I H P S D S E T R L N Q K F N D R A T L T V D K Y S S T A Y
91 I Q L S S P T S E D S A V Y Y C A R G E Y Y Y G I F D
```

2.3 Construction and expression of the bispecific single chain antibody CCR5xCD3

A schematic depiction of structure and mode of action of the CCR5xCD3 bispecific single chain antibody is shown in Fig 3. As described previously, the light and heavy variable domains were joined to a single-chain fragment using a (Gly4Ser1)3 linker and expressed in the periplasmic space of E. coli to test binding of the recombinant protein to CCR5.

Subsequently, the DNA sequence of the αCCR5 single-chain fragment was subcloned with BsrG1 and BspE1 into an eukaryotic expression vector (pEF-DHFR) that already contained a single-chain fragment directed against CD3 with a C-terminally attached tail of 6 histidine residues (Mack (1995) Proc. Natl. Acad. Sci. 92, 7021). The αCCR5 and αCD3 single-chain fragments were joined by a linker coding for Gly4Ser1 (see Fig 3).

The following order of the domains is chosen: VL(1)-VH(1)-VH(2)-VL(2), with (1) being the specificity against CCR5 and (2) the specificity against CD3.

The bispecific CCR5xCD3 antibody has the following nucleotide sequence, SEQ ID NO. 17:

```
   1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT ACACTCCGAT
  61 ATCGTGCTGA CCCAGTCTCC AGCCTCCCTA TCTGCATCTG TGGGAGAAAC TGTCACCATC
 121 ACATGTCGAG CAAGTGAGAA TATTTACAGT TATTTAGCAT GGTATCAGCA GAAACAGGGA
 181 AAATCTCCTC AACTCCTGGT CTATAATGCA AAAACCTTAA CAGAAGGTGT GCCATCAAGG
 241 TTCAGTGGCA GTGGATCAGG CACACAGTTT TCTCTGAAGA TCAACAGCCT GCAGCCTGAA
 301 GATTTTGGGA ATTATTTCTG TCAACATCAT TATGATACTC CTCGGACGTT CGGTGGAGGG
 361 ACCAAGCTCG AGATCAAAGG TGGTGGTGGT TCTGGCGGCG GCGGCTCCGG TGGTGGTGGT
 421 TCTCAGGTCC AACTGCAGCA GCCTGGGGCT GGGAGGGTGA GGCCTGGAGC TTCAGTGAAG
 481 CTGTCCTGCA AGGCTTCTGG CTACTCCTTC ACCAGTTACT GGATGAACTG GGTGAAGCAG
 541 AGGCCTGGAC AAGGCCTTGA GTGGATTGGC ATGATTCATC CTTCCGATAG TGAAACTAGG
 601 TTAAATCAGA AGTTCAACGA CAGGGCCACA TTGACTGTTG ACAAATATTC CAGCACAGCC
 661 TATATACAAC TCAGCAGCCC GACATCTGAG GACTCTGCGG TCTATTACTG TGCAAGAGGA
 721 GAATATTACT ACGGTATATT TGACTACTGG GGCCAAGGGA CCACGGTCAC CGTCTCCTCC
 781 GGAGGTGGTG GATCCGATAT CAAACTGCAG CAGTCAGGGG CTGAACTGGC AAGACCTGGG
 841 GCCTCAGTGA AGATGTCCTG CAAGACTTCT GGCTACACCT TTACTAGGTA CACGATGCAC
 901 TGGGTAAAAC AGAGGCCTGG ACAGGGTCTG GAATGGATTG GATACATTAA TCCTAGCCGT
 961 GGTTATACTA ATTACAATCA GAAGTTCAAG GACAAGGCCA CATTGACTAC AGACAAATCC
1021 TCCAGCACAG CCTACATGCA ACTGAGCAGC CTGACATCTG AGGACTCTGC AGTCTATTAC
1081 TGTGCAAGAT ATTATGATGA TCATTACTGC CTTGACTACT GGCGCCAAGG CACCACTCTC
1141 ACAGTCTCCT CAGTCGAAGG TGGAAGTGGA GGTTCTGGTG GAAGTGGAGG TTCAGGTGGA
1201 GTCGACGACA TTCAGCTGAC CCAGTCTCCA GCAATCATGT CTGCATCTCC AGGGGAGAAG
1261 GTCACCATGA CCTGCAGAGC CAGTTCAAGT GTAAGTTACA TGAACTGGTA CCAGCAGAAG
1321 TCAGGCACCT CCCCCAAAAG ATGGATTTAT GACACATCCA AAGTGGCTTC TGGAGTCCCT
1381 TATCGCTTCA GTGGCAGTGG GTCTGGGACC TCATACTCTC TCACAATCAG CAGCATGGAG
```

```
1441 GCTGAAGATG CTGCCACTTA TTACTGCCAA CAGTGGAGTA GTAACCCGCT CACGTTCGGA
1501 GCTGGGACCA AGCTGGAGCT GAAACATCAT CACCATCATC ATTAG
```

The bispecific CCR5xCD3 antibody has the following protein sequence, SEQ ID NO. 18:

```
  1 D I V L T Q S P A S L S A S V G E T V T I T C R A S E N I Y
 31 S Y L A W Y Q Q K Q G K S P Q L L V Y N A K T L T E G V P S
 61 R F S G S G S G T Q F S L K I N S L Q P E D F G N Y F C Q H
 91 H Y D T P R T F G G G T K L E I K G G G G S G G G G S G G G
121 G S Q V Q L Q Q P G A G R V R P G A S V K L S C K A S G Y S
151 F T S Y W M N W V K Q R P G Q G L E W I G M I H P S D S E T
181 R L N Q K F N D R A T L T V D K Y S S T A Y I Q L S S P T S
211 E D S A V Y Y C A R G E Y Y Y G I F D Y W G Q G T T V T V S
241 S G G G G S D I K L Q Q S G A E L A R P G A S V K M S C K T
271 S G Y T F T R Y T M H W V K Q R P G Q G L E W I G Y I N P S
301 R G Y T N Y N Q K F K D K A T L T T D K S S S T A Y M Q L S
331 S L T S E D S A V Y Y C A R Y Y D D H Y C L D Y W R Q G T T
361 L T V S S V E G G S G G S G G S G G S G G V D D I Q L T Q S
391 P A I M S A S P G E K V T M T C R A S S S V S Y M N W Y Q Q
421 K S G T S P K R W I Y D T S K V A S G V P Y R F S G S G S G
451 T S Y S L T I S S M E A E D A A T Y Y C Q Q W S S N P L T F
481 G A G T K L E L K H H H H H H *
```

The bispecific antibody was expressed in DHFR-deficient CHO cells and purified from the culture supernatant by affinity chromatography on immobilized Ni2+ ions (Hochuli (1988) Biotechnology 6, 1321-1325; Ni-NTA, Qiagen).

In summary, for the construction of bispecific antibodies, for example the single-chain technique may be used (Mack et al. (1995) Proc. Natl. Acad. Sci. U S A, 92:7021-7025; Mack et al. (1997) J. Immunol. 158:3965-3970). In this case, as shown schematically in Figures 3 (top), the variable domains of the light (VL) and the heavy (VH) immunoglobulin chains of two different antibodies are fused in a particular order, optionally a histidine chain of 6 x His is attached in addition. The fusion is effected on a DNA basis so that a protein chain with four different variable domains is formed after expression (cf. Figures 3 (top)). The attached histidine chain enables a simple and efficient purification via immobilized Ni ions in one step. Figure 3 (top) shows a preferred embodiment of the bispecific antibody binding to the CD3 antigen on the surface of the effector cell and the human CCR5 on the surface of leukocytes as target cells.

Subsequently, a single-chain antibody with a specificity is generated by means of fusion PCR by inserting a linker of $(Gly_4Ser_1)_3$ between the two variable antibody domains. In a further fusion PCR, the antibody fragment against CCR5 is fused to the already published antibody fragment against CD3, with a linker consisting of Gly_4Ser_1 is inserted (cf. Mack et al. supra).

In order to express the bispecific antibody, the corresponding DNA sequence is subcloned in a eukaryotic expression vector (e.g. PEF-DHFR, Mack et al. (1995) PNAS, supra) and transfected in DHFR-deficient CHO cells by means of electroporation. The bispecific antibody is purified from the supernatant of stably transfected CHO cells by means of affinity chromatography at Ni-NTA, with elution taking place by lowering the pH value. Subsequently, the pH is adjusted and the protein is adjusted to a suitable concentration. Overall purification yield was approx. 900 µg/l culture supernatant. SDS-PAGE showed a single band of approx. 60 kD under reducing and non-reducing conditions without any detectable proteolysis or degradation of the protein (Fig. 4).

Example 3: Expression and purification of a chemokine-toxin fusion protein

A schematic depiction of structure and mode of action of the RANTES-PE38 chemokine-toxin fusion protein is shown in Fig 5. A PCR fragment of RANTES, generated with the primers P1 and P2, was subcloned with StuI and SalI into a vector for periplasmic expression in E. coli (Mack (1995) Proc. Natl. Acad. Sci. 92, 7021). The restriction site StuI had previously been introduced at the 3' terminus of the OmpA signal sequence. The DNA of a truncated version of Pseudomonas exotoxin A (PE38; Theuer (1993) Cancer Res. 53, 340), was amplified by PCR with Pfu-polymerase using the primers P3 and P4 and subcloned with BspE1 and Hind III into the vector that already contained the cDNA of RANTES. Primer P4 also added a tail of 6 histidine residues at the 3' terminus of PE38. During the periplasmic expression the OmpA signal sequence is cleaved off such that the recombinant protein starts with the first aminoacid of RANTES. The C-terminally attached tail of 6 histidine residues allowed purification by affinity chromatography on Ni-NTA (Qiagen).

List of primers:

SEQ ID NO. 19: 5′ AAAGGCCTCCCCATATTCCTCGGA

SEQ ID NO. 20: 5′ AAAGTCGACTCCGGACATCTCCAAAGAGTTGATGTAC

SEQ ID NO. 21: 5′ AATCCGGAGGCGGCAGCCTGGCCGC

SEQ ID NO: 22: 5′ GGGAAGCTTAGTGATGGTGATGGTGATGCTTCAGGTCCTCGCGCGG

As described in the above the DNA sequence of RANTES was fused with the sequence of a truncated version of the Pseudomonas exotoxin A (PE38) (Theuer (1993) Cancer Res. 53, 340). In a first version of the construct a Gly-Ser linker was spaced between RANTES and PE 38. However this resulted in a considerable proteolytic degradation of the fusion protein during expression in E. coli (data not shown). In an attempt to stabilize the construct the linker and the fist three aminoacids of PE38 were removed. The new fusion protein showed no proteolysis during expression in the periplasmic space of E. coli as demonstrated by SDS-PAGE (Fig. 6 left panel) and Western-blot (Fig. 6 right panel). The corresponding constructs are depicted in SEQ ID NOs: 23 and 24, respectively.

Example 4: Binding of the bispecific antibody to the target antigens CCR5 and CD3

Binding of the bispecific single-chain antibody to CHO cells or PBMC was determined by FACS-analysis (Fig. 7 to 9). The cells were incubated with the bispecific antibody for 60 min on ice followed by an antibody against 6xHis (Dianova, Hamburg, Germany) and a PE-conjugated polyclonal rabbit-anti mouse F(ab)2 fragment (R439, Dako, Hamburg, Germany). As the bispecific antibody would also bind to CCR5, we performed the analysis with PBMC that lack expression of CCR5 due to a homozygous 32 basepair deletion in the CCR5 alleles. The antibody showed good binding to a subpopulation of lymphocytes. Co-staining with antibodies against CD4 and CD8 identified this subpopulation as CD4 and CD8 positive T lymphocytes (Fig. 7). In addition, the bispecific antibody competed with the monoclonal CD3 antibody OKT-3 for binding to T cells (data not shown).

Binding of the bispecific antibody to CCR5 was demonstrated on CCR5 overexpressing CHO cells and human monocytes (Fig. 8 and 9). The antibody showed excellent binding to CCR5 transfected CHO cells (Fig. 8) and cultured monocytes (Fig 9), while no

binding was detectable on CHO cells transfected with CXCR4 or on cultured monocytes from a donor with a homozygous CCR5-Δ32/Δ32 deletion. Overnight cultivation of monocytes induces expression of CCR5 on wild-type monocytes, while monocytes from donors with a homozygous CCR5-Δ32/Δ32 deletion fail to express CCR5. Moreover we have shown that the CCR5 signal detectable with the bispecific antibody on cultured monocytes could be reduced to values below 15 % by preincubation of monocytes for 30 min at 37°C with AOP-RANTES (data not shown) that is known to efficiently induce internalization of CCR5 and reduce binding of CCR5 antibodies (25).

Example 5: Downmodulation of chemokine receptors

5.1 Downmodulation of CCR5 with mAb MC-1 against CCR5

The effect of MC-1 one on the surface expression of human CCR5 was measured. For comparison a different monoclonal antibody MC-4 against CCR5 was used. CHO-CCR5 cells were incubated with various concentrations of antibody MC-1 and MC-4 for 30 min. at 37°C. Cells were placed on ice and stained with MC-1 and MC-4 respectively at a concentration of 15 ug/ml for one hour on ice, followed by detection with a secondary antibody (rabbit anti-mouse FITC, F313 from DAKO). Analysis was performed on a FACSCalibur. Incubation with MC-1 at 37oC for 30 min resulted in a downmodulation of human CCR5 by 40% at a concentration of 10 ug/ml (Fig. 10).

5.2 Downmodulation of CCR5 by chemokine-toxin

The fusion of RANTES to the N-terminus of a truncated version of the Pseudomonas exotoxin A is supposed to result in specific binding of the construct to cells expressing RANTES receptors such as CCR5, CCR1 and CCR3. Internalization of the chemokine receptors upon binding of the modified toxin would enhance the cellular uptake and cytotoxic activity of the construct (Fig. 5 lower panel). We therefore analyzed whether RANTES-PE38 is able to internalize CCR5 from the surface of primary monocytes and T cells (Fig. 11, open symbols). Internalization of CCR5 would indicate that the construct is able to bind to CCR5 and that RANTES remains functionally active after fusion to PE38. As shown in Fig. 11 the construct is able to internalize CCR5 from the surface

of monocytes and lymphocytes. Unmodified RANTES served as positive control and was somewhat more efficient than RANTES-PE38 (Fig 11, closed symbols).
PBMC were incubated for 30 min at 37°C with various concentrations of RANTES or RANTES-PE38 diluted in RPMI with 10% FCS in a volume of 100 µl. Medium alone was used as control. The cells were then stained on ice for surface CCR5 expression using the monoclonal antibody MC-1 or medium as negative control followed by the PE-conjugated anti-mouse antibody R439. The FACS-analysis was performed on a FACSCalibur (Becton Dickinson) and CellQuest software. Lymphocytes and monocytes were distinguished by their forward and sideward light scatter properties and expression of CD14, CD4 and CD8. Relative surface CCR5 expression was calculated as [mean channel fluorescence (exp.) - mean channel fluorescence (negative control)] / [mean channel fluorescence (medium) - mean channel fluorescence (negative control)].

Example 6: Depletion of cells with CCR5xCD3 antibody and RANTES-PE38

6.1 CCR5 specific depletion of monocytes from cultured PBMCs

PBMC from CCR5-wildtype (WT) or CCR5 deficient (Δ32/Δ32) donors were incubated over night to induce expression of CCR5 on monocytes. Cultured PBMC were incubated with different concentrations of purified αCCR5-αCD3 bispecific antibodies or medium as control for 20 h. Surviving cells were analyzed on a FACSCalibur and counted.
In order to test the ability of the αCCR5-αCD3 bispecific single-chain antibody to deplete CCR5 positive primary cells, we incubated human PBMC with the antibody (Fig. 12). Prior to incubation the PBMC were cultured overnight to upregulate CCR5 expression on monocytes. By retargeting cytotoxic T cells the bispecific antibody depleted the majority of monocytes within 20 h in a concentration dependent manner (Fig. 13) with an almost complete elimination of CCR5 positive cells at concentration of 10 ng/ml. To verify that the depletion of monocytes was due to their induced expression of CCR5, the same experiment was performed with PBMC from a donor with a homozygous 32 bp deletion in the CCR5 allele that prevents surface expression of CCR5. No depletion of CCR5 deficient monocytes was detectable after 20 h indicating that the depletion of

cells with the bispecific antibody is restricted to monocytes that express CCR5 (Fig. 12), compare lower right panel to upper right panel, left panels serve as negative controls. Monocytes (Mo) and lymphocytes (Ly) were identified by their forward and sidewards light scatter properties. Monocytes appear in the lower left quadrant see arrows.

6.2 Depletion of monocytes and T lymphocytes from the synovial fluid of patients with arthritis

Freshly drawn synovial fluid of patients with arthritis were incubated with different concentrations of purified αCCR5-αCD3 bispecific antibodies or medium as control for 20 h. Surviving cells were analyzed on a FACSCalibur and counted.

The bispecific single-chain antibody could potentially be applied to deplete CCR5 positive T cells and monocytes from the inflamed joints of patients with arthritis. Therefore determined the depletion of CCR5 positive cells from the synovial fluid of patients with various types of arthritis was determined. It was shown previously that the majority of T cells and monocytes in the inflamed synovial fluid express CCR5 (Mack (1999) loc. cit.). In synovial samples obtained before depletion experiments it was confirmed by FACS analysis that the majority of lymphocytes and monocytes express CCR5, while no expression of CCR5 was detectable on granulocytes (data not shown). For the depletion experiments the synovial fluid was incubated ex vivo with different concentrations of the bispecific antibody for 20 h (Fig. 14). The synovial fluid was incubated immediately after puncture without any preparation to ensure that the conditions in vitro resemble most closely the situation in vivo when the antibody would be present within inflamed joints. As shown in Fig. 14 the bispecific antibody induced a depletion of the majority of lymphocytes and monocytes from the synovial fluid, while granulocytes that do not express CCR5 remained unaffected. A representative FACS analysis of the depletion of monocytes and lymphocytes in synovial fluid at a concentration of 0.5 ug/ml CCR5xCD3 is shown in Fig 15. Only the CCR5 negative neutrophils (PMN: polymorpho-nuclear cells) are unaffected by the bispecific antibody.

Antibodies were incubated with synovial fluid for one or several days. After 24 hours, the CCR5 positive lymphocytes and monocytes have already almost disappeared. When the medium is controlled after longer incubation, the monocytes have differenti-

ated into macrophages which are visible at the bottom of the culture flask. After an appropriate incubation with the bispecific antibody, no macrophages are visible.

A corresponding result can be obtained when cultivated PBMC are incubated with the bispecific antibody as described above. In this case, there is an almost complete depletion of CCR5 positive monocytes and an almost complete depletion of CCR5 positive T-lymphocytes. The depletion of CCR5 positive T-cells and monocytes is shown.

The results show that the construct of the present invention is capable of destroying CCR5 positive monocytes. This applies to both monocytes from the joint aspirate and blood monocytes which express CCR5 when being differentiated into macrophages. Depletion of the monocytes/macrophages takes place within a few hours (< 24 hrs). In particular the depletion of monocytes/macrophages in the joint is of great advantage in therapy since it is these cells that are mainly responsible for the joint destruction. Moreover, for the activation of T-lymphocytes an interaction with macrophages is also required so that, at the same time, the function of the T-lymphocytes is suppressed.

In addition to the depletion of monocytes/macrophages, a considerable reduction in the number of CCR5 positive T-lymphocytes could be observed.

6.3 Comparison of the efficacy of the bispecific antibody CCR5xCD3 versus monoclonal antibodies

The efficacy of the αCCR5-αCD3 bispecific single-chain antibody in depleting CCR5 positive monocytes was compared with the efficacy of two unmodified monoclonal antibodies. PBMC from two different donors (F and N) were cultured overnight and then incubated for 24 h with medium, the bispecific single-chain antibody (125 ng/ml), MC-1 (5µg/ml) and MC-5 (5µg/ml). The monoclonal antibody MC-1, the parental antibody for the bispecific single-chain antibody has the isotype mouse IgG-1 and the antibody MC-5 has the isotype IgG-2a. The cells were completely recovered and analyzed by FACS to quantify surviving monocytes and lymphocytes.

Fig. 16 shows that surprisingly only the bispecific antibody was able to considerably deplete CCR5 positive monocytes, while the unmodified monoclonal antibodies were

largely ineffective even when used in a 40 fold excess over the bispecific antibody CCR5xCD3. By FACS analysis using forward and sideward light scatter properties of lymphocytes and monocytes demonstrates that only the CCR5xCD3 bispecific antibody but not the monoclonal antibodies are capable of depleting cultured monocytes (Fig 17 compare right upper panel to lower panels).

6.4 Depletion of chemokine receptor expressing cells with RANTES-PE38

CHO cells expressing CCR5 or CXCR4 were grown to subconfluence on 24 well culture plates and incubated with different concentrations of purified RANTES-PE38 or medium as control. After 40 hours the adherent and non-adherent cells were recovered and analyzed by FACS to measure the percentage of dead cells. It was previously established that dead (propidium iodide positive) CHO cells can be identified by their light scatter properties.

It was further analyzed the cytotoxic activity of RANTES-PE38. For that purpose we incubated CHO cells expressing human CCR5, murine CCR5 and human CXCR4 with various concentrations of the chemokine-toxin or medium. No surviving (adherent) human or murine CCR5 positive CHO cells were detectable by light microscopy after 40 h incubation with as little as 10 nM RANTES-PE38. In contrast regular growth and survival was observed when the CCR5 positive cells were incubated with medium or when CXCR4 positive CHO cells were incubated with equal concentrations of the chemokine-toxin (data not shown). To quantify the percentage of dead cells the adherent and non-adherent cells were analyzed by FACS. It was previously established that living and dead CHO cells can be identified by their light scatter properties, the position of dead and alive cells is indicated by arrows (Fig 18). As shown in Fig. 18 no cytotoxic effect of RANTES-PE38 was seen on CHO cells expressing CXCR4, while CHO cells expressing human CCR5 were completely killed by 10 nM RANTES-PE38.

These experiments show that RANTES-PE 38 is able to internalize CCR5 from the surface of cells and induces depletion of cells expressing the RANTES receptors hCCR5 or mCCR5. The inactivity of the construct against CXCR4 positive CHO cells demonstrates that the cytotoxic activity of the construct is restricted to cells that express specific chemokine receptors.

Example 7: Virus infection assay with stable transfected cells

GHOST 34 CCR5 cells are derived from HOS/CD4 cells stably expressing CCR5 and were provided by Dan Littman (Skirball Institute, New York). 2.5 x 104 cells in 48-well trays were exposed to 100 µl of chemokine at appropriate dilution for 30 min at 37°C. 100 µl of the NSI, CCR5-dependent HIV-1 strain, SF162 was added at 1000 focus forming units/ml (FFU/ml) and the cells incubated for a further 3 h. The cells were then washed and incubated in medium containing the appropriate chemokine for 4 days before fixing, staining in situ for p24 production and estimating foci of infection as previously described.

EXAMPLE 8: Concentration dependent binding of CCR5xCD3 to CCR5 expressing CHO cells

Chinese hamster ovary cells stably transfected with CCR5 (CCR5+CHO) were used as target cells for binding studies of bispecific scFv CCR5xCD3 (as described in Example 2 and Fig. 3). These cells were negative for CD3 and >95% positive for CCR5 as evaluated by binding assays with the parental antibody MC-1 (as described in Example 5 and Fig. 10). Binding was evaluated by a flow cytometry based binding assay.
$4x10^5$ CCR5+CHO cells were resuspended in 50 µl FACS buffer (PBS with 1% fetal calf serum (FCS) and 0,05% sodium azide) containing different dilutions of scFV CCR5xCD3 ranging from 20 µg/ml to 19.5 ng/ml. Cells were incubated in a 96 well microtiter plate for 30 minutes at 4°C. Cells were washed twice with FACS buffer and incubated for 45 minutes at 4°C with 20 µg/ml anti-His-Tag monoclonal antibody (Dianova). Specifically bound scFV CCR5xCD3 was detected with a monoclonal goat anti-mouse IgG F(ab')2 -PE conjugated antibody (Dianova). After washing, the cells were analysed in a flow cytometer (FACSCalibur, Becton Dickinson) using the CellQuest software (Becton Dickinson) to calculate the median values of the fluorescence intensities of the different concentration samples. Nonlinear regression analysis was performed with GraphPad Prizm (Version 3.02). Concentration dependent binding of scFv CCR5xCD3 to CCR5 expressing CHO cells was observed with a K_D value of 0,86 µg/ml (Fig. 20).

EXAMPLE 9: Cytotoxic activity of CCR5xCD3 with primary T lymphocytes as effector cells

The capacity of scFv CCR5xCD3 (as described in Example 2 and Fig. 3) to mediate cytotoxicity to CCR5-positive cells was tested using stably transfected CCR5+CHO as target cells and CD3 positive T-lymphocytes derived from peripheral blood as effector cells. For detection of cytotoxicity, a FACS based assay was performed.

CD3+ T-cells (include CD4+ and CD8+ cells) were isolated from peripheral blood by negative selection using a human T cell enrichment column (R&D Systems). For this purpose, PBMC were prepared by standard Ficoll-Hypaque density gradient separation and applied to the column. B cells and monocytes were bound to the column matrix, while T cells were eluated. The enriched T cells were washed in medium and used as effector cells.

For discrimination of target cells from effector cells by flow cytometry, CCR5+CHO cells were labeled with the aliphatic membrane dye PKH26 (Sigma) in a final concentration of 12 µM. $0.5x10^5$ labeled CCR5+CHO cells and $2.5x10^5$ CD3+ T-cells were seeded in a 96-well microtiter plate in a effector:target ratio of 5:1. 100 □l dilutions of scFv CCR5xCD3 ranging from 320 ng/ml to 0.3 pg/ml were incubated with the cells for 16 hours at 37°C in a humified atmosphere at 5% CO_2. Subsequently cells were centrifuged for 3 minutes at 600xg, and the cell pellets were resuspended in 200 µl FACS buffer (PBS, 1%FCS, 0.05% sodium azide). After staining with 1 µg/ml propidium iodine (PI), cells were analyzed in duplicate in a flow cytometer (FACSCalibur, Becton Dickinson).

In order to verify the specificity of scFv CCR5xCD3 mediated lysis, stably CXCR4 transfected CHO cells were used as negative control target cells. The cytotoxicity assay was performed under identical conditions as described for CCR5+CHO cells.

Specific lysis of CCR5+CHO cells was calculated using the CellQuest software (Becton Dickinson) and a nonlinear regression analysis was performed with GraphPad Prizm. A sigmoidal dose response curve was obtained (Fig. 21) revealing an EC50 value of 912 pg/ml. No cytotoxic effect of scFv CCR5xCD3 was observed using CXCR4+ CHO cells as target cells.

EXAMPLE 10: Cytotoxic activity of CCR5xCD3 with T cell clone CB15 as effector cells

The cytotoxic activity of scFv CCR5xCD3 (as described in Example 2 and Fig. 3) on CCR5-positive cells was also tested using the CD3 positive T-cell line CB15 (CD4+) as effector cells. For detection of cytotoxicity, a FACS based assay was performed with CCR5 transfected CHO cells (CCR5+CHO) as target cells.

CCR5+CHO cells were labeled with the aliphatic membrane dye PKH26 (Sigma) in a final concentration of 10 µM. Effector and target cells were incubated in a microtiter plate in a ratio of 10:1 with 100 µl of scFv CCR5xCD3 in dilutions ranging from 40 µg/ml to 0.15 ng/ml for 6 hours at 37°C in a humified atmosphere with 5% CO_2. Cells were centrifuged for 3 minutes at 600xg and the cell pellets were resuspended in 200 µl FACS buffer (PBS, 1%FCS, 0.05% sodium azide). Cells were stained with 1 µg/ml propidium iodine (PI) and analyzed in duplicate in a flow cytometer (FACSCalibur, Becton Dickinson).

Specific lysis of CCR5+CHO cells was calculated using the CellQuest software (Becton Dickinson) and a nonlinear regression analysis was performed with GraphPad Prizm. A sigmoidal dose response curve was obtained (Fig. 22) revealing an EC50 value of 12.8 ng/ml.

The results obtained with T cell clone CB15 as effector cells in bioactivity assay demonstrate that specific lysis mediated by scFv CCR5xCD3 is not restricted to the cytotoxic activity of CD8+ CTL but that CD4+ T cells are also involved in this process.

EXAMPLE 11: Epitope mapping of parental CCR5 specific monoclonal antibody MC-1 used for construction of scFv CCR5xCD3

Epitope of parental CCR5 specific monoclonal antibody MC-1 used for construction of scFv CCR5xCD3 (as described in Example 2 and Fig. 3) was mapped by flow cytometry using a panel of about 70 CHO-K1 cell lines stably expressing chimeric and point mutant receptors (Samson, J. Biol. Chem., 1997, 272, 24934-24941; Lee, J. Biol. Chem., 1999, 274, 9617-9626; Blanpain, J. Biol. Chem., 1999, 274, 34719-34727; Blanpain, Blood, 2000, 96, 1638-1645). Cells were incubated for 30 min on ice with mab MC-1, washed and stained with PE-conjugated anti-mouse Ig antibody (Sigma).

CHO-K1 cells expressing CCR2b were used as negative control. MC-1 was shown to recognize the first part of the second extracellular loop (ECL2) of the CCR5 molecule (data not shown). ECL2 ranges from aa 168-199 (RSQ KEGLHYTCSS HFPYSQYQFW KNFQTLKIV) and is located between the transmembrane regions 4 and 5 of CCR5 as described by Chen, J. Virol., 1997, 71, 2705-2714.

The amino acid sequences of human and rhesus macaque CCR5 differ in eight amino acids with two amino acid changes are situated at position aa 171 (K-->R) and aa 198 (I-->M) in the ECL2 (Chen, J. Virol., 1997, 71, 2705-2714). Due to these amino acid changes potential crossreactivity of MC-1 with the ECL2 of rhesus macaque CCR5 was analyzed with human and rhesus PBMCs in a FACS based assay. PBMC of both species were isolated by standard ficoll gradient centrifugation. $5x10^5$ cells were suspended in 50 µl FACS buffer and 50 µg/ml of MC-1 was added. After 30 min incubation at 4°C, the cells were washed and stained with goat anti-mouse IgG F(ab')2-PE conjugated monoclonal antibody (Dianova) for 30 min at 4°C in the dark. Cells were washed and analyzed in a flow cytometer (FACSCalibur, Becton Dickinson).

As shown in Fig. 23 MC-1 exclusively bound to human CCR5 but did not react with CCR5 derived from rhesus macaques. These data show that the epitope recognized by MC-1 is specific for human CCR5 and that lysin at position aa 171 and isoleucin at position aa 198 in human CCR5 sequence are essential for this specificity. Especially lysin at position aa 171 which is located in the first part of ECL2 contributes to the specific recognition of the human epitope of CCR5 by mab MC-1.

EXAMPLE 12: scFv CCR5xCD3 mediated reduction of virus production in HIV-1 infected monocytes

PBMC were prepared from fresh buffy coats of healthy donors by Ficoll density centrifugation and monocytes were isolated by over night adherence to culture flasks. Remaining PBL were removed and cultured separately at 37°C in a humidified atmosphere at 5% CO2.

Monocytes were seeded into a 48 well microtiter plate at a density of $5x10^4$ cells/well and infected with the M-tropic HIV-1 strain BaL (moi=1) overnight at 37°C in a humidified atmosphere at 5% CO_2. The virus was removed by washing and the monocytes

were further cultured with unstimulated PBL (15 x 10^4 per well) + scFv CCR5xCD3 (1 µg/ml) + AZT (75 µM) or with unstimulated PBL (15 x 10^4 per well) alone as negative control. 5 days post infection (p.i.) monocytes were washed and cultured in the absence of AZT or antibody. Supernatant was harvested on day 15 p.i. and HIV-1 replication was quantified by measurement of p24 in an ELISA. This experimental approach led to a reduction of virus replication of 75% in samples containing scFV CCR5xCD3 (75 ng/ml p24) compared to the control without scFv CCR5xCD3 (300 ng/ml p24).

Table I: Nucleosidal Reverse Transcriptase Inhibitors (nucleoside analogs, NRTI)

Substance	**Trade-name**	**Dosing Schedule**	**Common Side Effects and General Remarks**
Zidovudine (AZT)	Retrovir	300 mg, 2x daily	Initial gastrointestinal (GI) side effects, anemia, neutropenia, myopathy
Lamivudine (3TC)	Epivir	150 mg, 2x daily	Generally well tolerated. Effective against hepatitis
Zidovudine, Lamiduvine (AZT + 3TC)	Combivir	1 tablet 2x daily	Combination-tablet containing 300 mg AZT and 150 mg 3TC
Didanosine (ddI)	Videx	200 mg, 2x daily or 400 mg, 1x daily on an empty stomach (> 60 kg weight)	15% peripheral neuropathy, pancreatitis; avoid alcohol. Contents alcohol: could be given simultaneously with all NRTIs, Adefovir, Nevirapine, and Efavirence; Delavirdine and Indinavir should be given at least 1 hour before ddI; Nelfinavir to be given 1 hour after ddI.
Zalcitabine (ddC)	Hivid	0,375-0,75 mg, 3x daily	17-31% peripheral neuropathy in different studies; aphteous ulcerations
Stavudine (d4T)	Zerit	20-40 mg, 2x daily	Peripheral neuropathy (1-4% in earlier studies; 24% in 'expanded access' patients with CD4 > 50)
Abacavir (ABA)	Ziagen	300 mg, 2x daily	About 3% reaction for hypersensitivity: fever, indisposition, possibly transient rash, gastrointestinal side effects.

Table II: Protease Inhibitors

Substance	Trade-name	Dosing Schedule	Common Side Effects and General Remarks
Saquinavir (hard gelatine capsule, SQV-H)	Invirase	600 mg, 3x daily, take with high-fat meal	Well tolerated. Limited efficacy due to poor resorption.
Saquinavir (soft gelatine capsule, SQV-S)	Fortovase	1200 mg, 3x daily, take with high-fat meal (>28g)	Improved resorption compared to Invirase.
Ritonavir (RTV)	Norvir	600 mg, (6 cap./7.5 ml) 2x daily. Start with 300 mg, 2x daily, then increase within 10 days to 600 mg, 2x daily;	Nausea and numb lips for up to 5 weeks. Occasionally hepatitis. Not tolerated by up to 50% of the patients.
Indinavir (IDV)	Crixivan	800 mg, every 8 hours on an empty stomach or with snack (<2g fat)	Neural calculus with 6-8%; requires large liquid intake. Occasionally nausea and gastrointestinal side effects.
Nelfinavir (NFV)	Viracept	750 mg, 3x daily, or 1250 mg, 2x daily with meals	Often diarrhea, sometimes nausea.

Table III: Non-Nucleosidal Reverse Transcriptase Inhibitors (NNRTI)

Substance	Trade-name	Dosing Schedule	Common Side Effects and General Remarks
Nevirapine (NVP)	Viramune	200 mg, 1x daily	Transient skin, hepatitis, induced liver enzymes P450 3A4
Delavirdine (DLV)	Rescriptor	400 mg, 3x daily	Transient skin, suppresses P450 3A4
Efavirence (EFV)	Sustiva	600 mg, 1x daily in the evening	Initially dizziness, insomnia, momentary transient skin: Induces P450 3A4; avoid Claritithromycin.

Table IV: Chemokine receptors and chemokine ligands

Chemokine Receptors	**Chemokine Ligands**
CXCR3	I-TAC (CXCL11), IP-10 (CXCL-10), Mig (CXCL9)
CXCR4	SDF-1 (CXCL12)
CXCR5	BCA1 (CXCL13)
CCR1	MIP1alpha (CCL3), RANTES (CCL5), MCP-3 (CCL7), MCP-4 (CCL13), HCC1 (CCL14), LKN1 (CCL15)
CCR2	MIP1alpha (CCL3), RANTES (CCL5), MCP-1 (CCL2), MCP-2 (CCL8), MCP-3 (CCL7), MCP-4 (CCL13)
CCR3	RANTES (CCL5), MCP-2 (CCL8), MCP-3 (CCL7), MCP-4 (CCL13), eotaxin (CCL11), LKN1 (CCL15), MPIF-2 (CCL24) , eotaxin-3 (CCL26)
CCR4	TARC (CCL17), MDC (CCL22)
CCR5	MIP1alpha (CCL3), MIP1 beta (CCL4), RANTES (CCL5), MCP-2 (CCL8), MCP-3 (CCL7), MCP-4 (CCL13), eotaxin (CCL11)
CCR6	LARC (CCL20)
CCR7	ELC (CCL19), SLC (CCL21)
CCR8	I-309 (CCL1), MIP1 beta (CCL4), TARC (CCL17)
CCR9	TECK (CCL25)
XCR1	XCL1, XCL2
CCR10	CCL27, CCL28 (Wang (2000) J. Biol. Chem. 275, 22313-22323) CX3CR1: fractalkine (CXC3CL1)

CLAIMS

1. Use of an antibody and/or chemokine construct which binds to a chemokine receptor for the preparation of a pharmaceutical composition for the elimination of cells which are latently infected with a primate immunodeficiency virus.

2. The use of claim 1, wherein said primate immunodeficiency virus is a human immunodeficiency virus.

3. The use of claim 2, wherein said human immunodeficiency virus is HIV-1.

4. Use of an antibody and/or chemokine construct which binds to a chemokine receptor for the preparation of a pharmaceutical composition for the treatment, prevention and/or alleviation of inflammatory renal diseases, allergic reactions, inflammatory bowel diseases, multiple sclerosis, skin diseases, diabetes or transplant rejection.

5. The use of any one of claims 1 to 4, wherein said chemokine receptor is the chemokine receptor 5 (CCR5).

6. The use of claim 5, wherein said chemokine receptor 5 is the human CCR5.

7. The use of any one of claims 1 to 6, wherein said antibody construct is a bispecific antibody which binds to the chemokine receptor as a first antigen and a CD3 antigen of an effector cell as a second antigen.

8. The use of claim 7, wherein said bispecific antibody is a single chain antibody construct.

9. The use of claim 8, wherein said single chain antibody construct comprises V_L and V_H domains of a antibody specific for the chemokine receptor and V_H and V_L

domains of an antibody specific for a CD3 antigen.

10. The use of claim 9, wherein said antibody specific for the chemokine receptor is the murine anti-human CCR5 antibody MC-1.

11. The use of claim 9 or 10, wherein said V_L and V_H domains are arranged in the order V_L(MC-1)-V_H(MC-1)-V_H(CD3)-V_L(CD3).

12. The use of claim 11, wherein said V_L(MC-1) comprises the amino acid sequence as depicted in SEQ ID NO: 12, wherein said V_H(MC-1) comprises the amino acid sequence as depicted in SEQ ID NO: 16, wherein said V_H(CD3) comprises the amino acid sequence as depicted in SEQ ID NO: 26 and/or wherein said V_L(CD3) comprises in SEQ ID NO: 28.

13. The use of any one of claims 5 to 12, wherein said bispecific antibody comprises an amino acid sequence encoded by the nucleic acid sequence as depicted in SEQ ID NO: 17 or comprises the amino acid sequence as depicted in SEQ ID NO: 18.

14. The use of any one of claims 1 to 6, wherein said antibody construct is a bispecific antibody which binds to said chemokine receptor as a first antigen and a toxin as a second antigen.

15. The use of any one of claims 1 to 6, wherein said antibody construct is covalently bound to a toxin.

16. The use of any one of claims 1 to 6, wherein said antibody construct can, via a multimerization domain, be bound in vitro and/or in vivo to a second antibody construct which binds to a CD3 antigen and/or a toxin.

17. The use of any one of claims 1 to 6, wherein said chemokine construct is a fusion construct of a modified or an unmodified chemokine with a modified or an

unmodified toxin.

18. The use of any one of claims 1 to 6, wherein said chemokine construct can, via a multimerization domain, be bound in vitro and/or in vivo to an antibody construct which binds to a CD3 antigen and/or to a toxin.

19. The use of any one of claims 1 to 6, wherein said chemokine construct comprises a chemokine covalently bound to an antibody construct which binds to an antibody construct which binds to a CD3 antigen and/or which is a covalently bound to a toxin.

20. The use of any one of claims 1 to 6, wherein said antibody and/or chemokine construct is a heterominibody construct comprising at least an antibody and/or a chemokine which binds to a chemokine receptor.

21. The use of claim 20, wherein said heterominibody construct comprises at least one toxin.

22. The use of claim 20 or 21, wherein said heterominibody construct binds to the chemokine receptor and/or to a CD3 antigen of an effector cell.

23. The use of any one of claims 17 to 22, wherein said chemokine is selected from the group consisting of RANTES, MIP-1ß, MIP-1α, MCP-2 and MCP-3..

24. The use of any one of claims 15 to 19, 21 and 22, wherein said toxin is a truncated Pseudomonas exotoxin A.

25. The use of any one of claims 17 to 24 wherein said chemokine construct comprises a amino acid sequence as depicted in SEQ ID NO: 24 or as encoded by the nucleotide sequence as depicted in SEQ ID NO: 23.

26. The use of any one of claims 7 to 13, 16, 18, 19 and 22, wherein said CD3 anti-

gen is on the surface of an effector cell which is a T-cell.

27. An antibody construct as defined in any one of claims 5 to 16, 20, 21 and 22, wherein said construct comprises a binding site for CCR5 and a binding site for CD3.

28. A chemokine construct as defined in any one of claims 17 to 22, wherein said chemokine construct comprises RANTES and said toxin is a truncated Pseudomonas exotoxin A (PE38).

29. A polynucleotide encoding an antibody-construct as defined in any one of claims 5 to 12 or a chemokine construct as defined in claims any one of claims 17 to 19, 23 and 24, wherein said polynucleotide is
    (a) a polynucleotide comprising the nucleic acid molecule in particular encoding the polypeptide as depicted in SEQ ID NO: 18 or SEQ ID NO: 24;
    (b) a polynucleotide comprising the nucleic acid molecule as depicted in SEQ ID NO: 17 or SEQ ID NO: 23; or
    (c) a polynucleotide hybridizing under stringent conditions to the complementary strand of a polynucleotide of (a) or (b).

30. The polynucleotide of claim 29 which is DNA or RNA.

31. A vector comprising the polynucleotide of claim 29 or 30.

32. The vector of claim 31 which is an expression vector or a gene transfer vector.

33. A host transformed with the vector of claim 31 or 32.

34. A method of producing the antibody construct or the chemokine construct as defined in claim 29 comprising culturing the host of claim 33 and isolating the produced antibody construct or chemokine construct.

35. The antibody construct or the chemokine construct encoded by the polynucleotide of claim 29 or 30 or produced by the method of claim 34.

36. A composition comprising the polynucleotide of claim 29, the vector of claims 31 or 32, the host of claim 33, the antibody construct of claim 27 or 35 and/or the chemokine construct of claim 28 or 35.

37. The composition of claim 36 which is a pharmaceutical composition further comprising, optionally, a pharmaceutically acceptable carrier, diluent and/or excipient.

38. The composition of claim 36 or 37 which further comprises a medicament for the treatment of an immunological disorder or a medicament for anti-HIV treatment.

39. A method for treating, preventing and/or alleviating an immunological disorder or for the elimination of cells which are latently infected with a primate immunodeficiency virus comprising administering to a subject in need of such a treatment, alleviation and/or prevention an effective amount of the composition of any one of claims 36 to 38.

40. Use of the polynucleotide of claim 29 or 30, the vector of claim 31 or 32, the host of claim 33, the antibody construct of claims 27 or 35 and/or the chemokine construct of claim 28 or 38 for the preparation of a pharmaceutical composition for treating, preventing and/or alleviating an immunological disorder.

41. Use of the polynucleotide of claim 29 or 30, the vector of claim 31 or 32, the host of claim 33, the antibody construct of claims 27 or 35 and/or the chemokine construct of claim 28 or 38 for the preparation of a pharmaceutical composition for eliminating latently infected cells, wherein said cells are infected with a primate immunodeficiency virus.

42. The method of claim 39 or the use of claim 40, wherein said immunological dis-

order is selected from the group consisting of autoimmune diseases, allergic diseases, skin diseases, inflammatory diseases, diabetes, graft versus host disease and transplant rejections.

43. The method or the use of claim 42, wherein said autoimmune disease is selected from the group consisting of multiple sclerosis, type I diabetes, and rheumatoid arthritis.

44. The method or the use of claim 42, wherein said skin disease is selected from the group consisting of skin inflammation, atopic dermatitis and psoriasis.

45. The method or the use of claim 42, wherein said inflammatory disease is selected from the group consisting of inflammatory joint diseases, inflammatory renal diseases, inflammatory bowel diseases.

46. The method or the use of claim 45, wherein said inflammatory joint disease is (chronic) arthritis.

47. The method of claim 39 or the use of claim 41, wherein said infection with a primate immunodeficiency virus is a HIV-1 infection.

48. The method or the use of claim 47, wherein said composition is to be administered in combination with antiviral agents and/or in combination with drugs to be employed in AIDS management.

49. The method or the use of claims 47, wherein said drugs to be employed in AIDS management comprises drugs employed in HAART.

50. A kit comprising the polynucleotide of claim 29 or 30, the vector of claim 31 or 32, the host of claim 33, the antibody construct of claims 27 or 35 and/or the chemokine construct of claim 28 or 38.