Humanized Anti-CD4 Antibody with Immunosuppressive Properties

The invention relates to a humanized anti-CD4 antibody, and to its use for immunomodulation.

Autoimmune diseases as well as graft rejection result from an inappropriate immune response to tissue antigens: self antigens in the first case, and allograft antigens in the second one.

Autoimmune diseases include for instance rheumatoid arthritis, type I diabetes, multiple sclerosis, Crohn's disease, ulcerative colitis, atopic dermatitis, etc.

Conventional treatments for these immunological disorders involve immunosuppressive drugs. However these drugs induce a general immunosuppression, resulting in inhibition of not only the harmful functions of the immune system, but also the useful ones. As a consequence, they induce side effects, such as opportunistic infections.

As an alternative approach, it has been proposed to use immunosuppressive monoclonal antibodies (mAbs) against cell-surface molecules, in order to remove specific lymphocyte subsets (depleting antibodies) or to inhibit the function of a target surface molecule without killing the cell bearing it (nondepleting-antibodies).

It is generally agreed that $CD4^+$ T cells play a major part in initiating and maintaining autoimmunity. Accordingly, it has been proposed to use mAbs against $CD4^+$ T cells surface molecules, and in particular anti-CD4 mAbs, as immunosuppressive agents. Although numerous clinical studies confirmed the potential interest of this approach, they also raised several issues to be addressed in order to make anti-CD4 mAbs more suitable for use in routine clinical practice.

By way of example, B-F5 antibody (murine IgG1 anti-human CD4) was tested in different autoimmune diseases:

- in rheumatoid arthritis patients, several open studies suggested a positive clinical effect of B-F5 at a daily dose of at least 20 mg (Racadot et al. Clin Exp Rheumatol 10(4):365-74; 1992; Wendling et al., Clin Rheumatol, 11(4):542-7, 1992). However, the results observed in a placebo controlled trial with a daily dose of 20 mg for

10 days did not show a significant improvement (Wendling et al. J Rheumatol;25(8):1457-61, 1998).

- in psoriasis, an improvement in psoriatic lesions was observed following a treatment at a dose of 0.2 mg/kg/day to 0.8 mg/kg/day for 7 or 8 days (Morel et al. J Autoimmun., 5(4):465-77, 1992) ;

- in multiple sclerosis (MS) patients, some positive effects were observed after a 10 days treatment in patients with relapsing-remitting forms, some of who were relapse-free at the 6th month post-therapy (Racadot et al., J Autoimmun, 6(6):771-86, 1993) ; similar effects were observed by Rumbach et al. (Mult Scler;1(4):207-12, 1996);

- in severe Crohn's disease, no significant improvement was observed in patients receiving B-F5 at a dose of 0.5 mg/day/kg for 7 consecutive days or of 0.5 mg/day/kg on the first day (day 0) and of 1 mg/day/kg on days 1-6 (Canva-Delcambre et al., Aliment Pharmacol Ther 10(5):721-7, 1996);

- in prevention of allograft rejection, a modification of the biological parameters, indicating an action of B-F5 *in vivo* at a 30 mg/daily dose was reported. However, it was reported that B-F5 bioavailability was not sufficient to allow its use for prophylaxis of allograft rejection (Dantal et al. Transplantation, 27;62(10):1502-6, 1996).

It appears from the above that a first issue to be solved is the need of using high doses of mAb to obtain a clinical improvement. This may result *inter alia* from the poor accessibility to the mAb of the lymphocytes in the target tissues. The use of higher doses may result in an excessive action on blood lymphocytes, inducing unwanted side effects.

Another drawback of therapy with monoclonal antibodies in humans is that these antibodies are generally obtained from mouse cells, and provoke antimouse responses in the human recipients. This not only results in a lesser efficiency of the treatment and even more of any future

treatment with mouse monoclonal antibodies, but also in an increased risk of anaphylaxis.

This drawback can, in principle, be avoided by the use of humanized antibodies, obtained by grafting the complementarity-determining regions (CDRs) of a mouse monoclonal antibody, which determine the antigen-binding specificity, onto the framework regions (FRs) of a human immunoglobulin molecule. The aim of humanization is to obtain a recombinant antibody having the same antigen-binding properties as the mouse monoclonal antibody from which the CDR sequences were derived, and far less immunogenic in humans.

In some cases, substituting CDRs from the mouse antibody for the human CDRs in human frameworks is sufficient to transfer the antigen-binding properties (including not only the specificity, but also the affinity for antigen). However, in many antibodies, some FR residues are important for antigen binding, because they directly contact the antigen in the antibody-antigen complex, or because they influence the conformation of CDRs and thus their antigen binding performance.

Thus, in most cases it is also necessary to substitute one or several framework residues from the mouse antibody for the human corresponding FR residues. Since the number of substituted residues must be as small as possible in order to prevent anti-mouse reactions, the issue is to determine which amino acid residue(s) are critical for retaining the antigen-binding properties. Various methods have been proposed for predicting the more appropriate sites for substitution. Although they provide general principles that may be of some help in the first steps of humanization, the final result varies from an antibody to another. Thus, for a given antibody, it is very difficult to foretell which substitutions will provide the desired result.

The inventors have however attempted the humanization of mouse B-F5, and have succeeded in producing humanized B-F5 (hereinafter referred to as hB-F5) having the same CD4 binding properties than parent mouse B-F5.

Furthermore, they have found that, surprisingly, hB-F5 has an *in vivo* optimal immunosuppressive effect at far lower doses than those previously used with parent B-F5, and than those currently used with other anti-CD4 monoclonal antibodies.

Actually, the inventors have observed that hB-F5 provided an effective immunosuppression, reflected by a positive clinical effect in rheumatoid arthritis patients, when used in a 10 days treatment at a dose as low as 1 mg/day, and preferably at a dose of 5 mg every second day.

The present invention provides an humanized antibody (hB-F5) derived from mouse B-F5 MAb, wherein said hB-F5 antibody has V domains defined by the following polypeptide sequences:

- H chain V domain: EEQLVESGGGLVKPGGSLRLSCAASGFSFSDCRMYWLRQA PGKGLEWIGVISVKSENYGANYAESVRGRFTISRDDSKNTVYLQMNSLKTEDTAVYYCSAS YYRYDVGAWFAYWGQGTLVTVSS (SEQ ID NO: 1)
- L chain V domain: DIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSYIYWYQQ KPGQPPKLLIYLASILESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQHSRELPWTFG QGTKVEIK (SEQ ID NO: 2).

Generally, a hB-F5 antibody of the invention further comprises a human constant region (Fc). This constant region can be selected among constant domains from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and IgG4. Preferred constant regions are selected among constant domains of IgG, in particular IgG1.

The present invention also includes any fragment of a hB-F5 antibody comprising the V regions thereof. This comprises in particular Fab, Fab', $F(ab)'_2$, Fv and scFv fragments.

The invention also encompasses a polynucleotide selected among:

- a polynucleotide encoding a polypeptide of SEQ ID NO:1;
- a polynucleotide encoding a polypeptide of SEQ ID NO:2.

Preferably, said polynucleotide is selected among:

- a polynucleotide of SEQ ID NO:3;
- a polynucleotide of SEQ ID NO:4.

Polynucleotides of the invention can easily be obtained by the well-known methods of recombinant DNA technology and/or of chemical DNA synthesis.

A polynucleotide encoding the V domain of the H chain or of the L chain of a hB-F5 antibody may be fused with a polynucleotide coding for the constant region of a human H or L chain, for the purpose of expressing the complete H and L chains obtained in this way; a sequence coding a signal peptide allowing the secretion of the protein can also be added. These recombinant polynucleotides are also part of the invention.

The invention also provides expression cassettes wherein a polynucleotide of the invention is linked to appropriate control sequences allowing the regulation of its transcription and translation in a chosen host cell, and recombinant vectors comprising a polynucleotide or an expression cassette of the invention.

These recombinant DNA constructs can be obtained and introduced in host cells by the well-known techniques of recombinant DNA and genetic engineering.

The invention also comprises an host cell, transformed by a polynucleotide of the invention.

Useful host-cells within the framework of the present invention can be prokaryotic or eukaryotic cells. Among suitable eukaryotic cells, one will mention, by way of example, plant cells, cells of yeasts such as *Saccharomyces*, cells of insects such as *Drosophila*, or *Spodoptera*, and mammal cells such as HeLa, CHO, 3T3, C127, BHK, COS, etc....

The construction of expression vectors of the invention, and the transformation of host-cells can be made by the standard techniques of molecular biology.

A hB-F5 antibody of the invention can be obtained by culturing a host cell containing an expression vector comprising a nucleic acid sequence encoding said antibody,

under conditions suitable for the expression thereof, and recovering said antibody from the host cell culture.

The present invention also comprises a therapeutic composition comprising a hB-F5 antibody of the invention or a fragment thereof, as defined above.

Preferably, said composition is a composition for parenteral administration, formulated to allow the administration of a dose of from 0.1 to 10 mg, advantageously of from 1 to 5 mg of hB-F5.

More specifically, the invention encompasses the use of a hB-F5 antibody of the invention or a fragment thereof, for preparing an immunosuppressive composition. Said immunosuppressive composition is useful in particular for the treatment or prevention of diseases such as graft rejection, graft-versus-host reaction or host-versus-graft reaction, or autoimmune diseases including for instance myocarditis, diabetes mellitus, psoriasis, lupus erythematosus, Crohn disease, multiple sclerosis, rheumatoid arthritis, etc .

Moreover, the inventors have found out that hB-F5 was able to activate a particular subset of T CD4+ cells, namely CD4+CD25+ cells.

$CD25^+CD4^+$ regulatory T cells (Treg cells) constitute 5-10% of peripheral $CD4^+$ T cells. They were first described in 1995 by Sakaguchi et al. (J. Immunol., 155:1151-1164) as regulatory cells in mice. When activated, these cells are able to suppress both CD4+ and CD8+ T cell activation and proliferation. Later, CD25+CD4+ suppressor T cells have also been found in humans (Jonuleit et al., J. Exp. Med.193, 1285-1294, 2001; Levings et al., J. Exp. Med. 193, 1295-1302, 2001; Dieckmann et al., J. Exp. Med. 193, 1303-1310 2001). Numerous articles have been published describing the immunosuppressive role of these cells in different autoimmune disease models and *in vitro* systems (for review, see for instance Shevach, J. Exp. Med., 193, 11, 41-46, 2001). *Ex vivo* activated $CD4^+CD25^+$ Treg cells have also been shown to be effective at preventing graft-versus-host disease (Taylor et al., Blood, 99, 3493-3499, 2002; Cohen et al., J. Exp. Med. 196, 401-406, 2002; Hoffmann et al., J.

Exp. Med. 196, 389-399, 2002). Thus, providing means for activating $CD4^+CD25^+$ Treg cells is of great interest.

The invention also relates to the use of the hB-F5 antibody of the invention, or of the parent antibody B-F5, to activate *in vitro* $CD25^+CD4^+$ regulatory T cells.

Preferably, the hB-F5 antibody of the invention is added to the $CD25^+CD4^+$ regulatory T cells at a concentration of 1µg/ml from 10µg/ml.

The present invention will be further illustrated by the following additional description, which refers to examples illustrating the properties of hB-F5 antibodies of the invention. It should be understood however that these examples are given only by way of illustration of the invention and do not constitute in any way a limitation thereof.

EXAMPLE 1: CONSTRUCTION OF HUMANIZED B-F5

Design of Humanized B-F5 V_H and V_K Regions

DNA sequences encoding mouse B-F5 V_H and V_k regions are respectively shown in Figure 1 and Figure 2 and under sequence identifiers SEQ ID NO:5 and SEQ ID NO:6. The human V_H and V_K on which the mouse CDRs are grafted were selected by searching databases for human V_H most like the original mouse B-F5 V_H and V_K. V_H region of a human antibody (M26; Accession Number A36006) had the highest homology with B-F5 V_H. V_K region of another human antibody (FK-001; NAKATANI *et al.*, *Biotechnology*, 7 (1989), 805-810)) had the highest homology with B-F5 V_K.

Two types of V_K differing between them in that the 4^{th} residue was Leucine or Methionine were constructed and designated as L4L and L4M. Two types of V_H differing between them in that the 37^{th} amino acid residue was Leucine or Valine, were constructed and designated as H37L and H37V. The alignment of the polypeptide sequences of B-F5, FK-001, L4L, and L4M is shown in Figure 3. The alignment of the polypeptide sequences of B-F5, M26, H37L, and H37V is shown in Figure 4. The FR residues previously reported to be important for the packing of CDRs (Chothia et al., Nature,

342(1989), 877; Foote et al., J. Mol. Biol., 224(1992), 487) are boxed.

By combining these V_H and $V\kappa$, 4 versions of V regions were designed.

Expression of humanized B-F5

The subsequent steps for production of humanized B-F5 were the same as those disclosed in US Patent 5,886,152 for humanized B-B10.

Briefly, expression plasmids for the H chain (V_H humanized region fused to the constant region of a human γ-1 chain (TAKAHASHI *et al., Cell*, 29 (1982), 671-679)) and the L chain ($V\kappa$ humanized region fused to the constant region of FK-001 κ chain) of humanized B-F5 were constructed separately. In these plasmids, the expression of humanized B-F5 is driven by the promoter/enhancer of the gene of human monoclonal IgM, FK-001. Figure 5 and 6 respectively show the fragments of the plasmids encoding the V_H and V_K regions of humanized BF-5. The sequences encoding the V region are underlined and the corresponding polypeptide sequences are indicated above the nucleotide sequence. Both plasmids and pSV2neo were simultaneously introduced into mouse myeloma Sp2/0 (ATCC CRL-1581) using Lipofectin™. Transfectomas producing human IgG were selected by ELISA, using an anti-human IgG (γ chain) antibody and an anti-human Ig κ chain antibody.

EXAMPLE 2 : CHARACTERISATION OF THE DIFFERENT VERSIONS OF HUMANIZED B-F5

Estimation of CD4 binding activity

Culture supernatants of transfectomas producing the four versions of hB-F5 were collected, and concentrated. The different antibodies were purified from culture supernatants by affinity chromatography using protein A Sepharose and assessed for their CD4 binding activity by measuring, by means of competitive ELISA, their inhibitory activities against the binding of biotinylated mB-F5 to soluble CD4 coated on microtiter plates. Incubation time is 2 hours for 37°C and overnight for 4°C.

The relative binding activities of hB-F5s (binding activity of mB-F5 was taken as 100%) are shown in Table I below

Table I

Antibody	Temp (°C)	Relative binding activity (% of mB-F5)
H37L/L4L	4	80
	37	30
H37L/L4M	4	80
	37	30
H37V/L4L	4	10-20
	37	10
H37V/L4M	4	10-20
	37	10

From the results shown in Table I, it appears that the 37th residue of V_H, Leucine, is critical to maintain CD4 binding activity of hB-F5 because the CD4 binding activity is several-fold reduced by conversion of ^{37}Leu to ^{37}Val. On the contrary, the 4th residue of Vκ is found to be not so important for the CD4 binding activity. As the structural difference between ^{37}Leu and ^{37}Val of V_H is not clearly demonstrated by molecular modeling, the superiority of H37L to H37V in CD4 binding activity was unexpected.

H37L/L4L and H37L/L4M were chosen for evaluating the *in vitro* biological activities.

Investigation of the in vitro biological activities of humanized B-F5

The *in vitro* biological activities of mouse B-F5 and humanized B-F5s (H37L/L4M IgG1 and H37L/L4L IgG 1) were evaluated. Humanized B-F5s of IgG2 type (H37L/L4M IgG2 and H37L/L4L IgG2) were also tested.

The *in vitro* biological activities of mB-F5 and the four types of hB-F5s were evaluated using peripheral blood mononuclear cells (PBMCs) from healthy donors. PBMCs were activated by ConA (2.5 µg/ml, 3 days) or PPD (10 µg/ml, 4 days) in the presence of murine or hB-F5s, and were monitored for their proliferative responses by ^{3}H-thymidine incorporation.

The results are shown in figures 7 and 8. Murine and hB-F5s could moderately inhibit ConA-induced proliferation, but the activities varied from antibody to

antibody and/or from donor to donor (Figure 7). Also, murine and hB-F5s were able to inhibit Ag-specific PBMC proliferation induced by PPD (Figure 8).

IgG1 type of hB-F5 inhibited PPD-induced proliferation more effectively (as high as 70% inhibition, Fig. 7 and 8) than mB-F5. IgG1 type seemed to be more effective than IgG2 type of which inhibitory activity was almost the same as mB-F5. For IgG1 type, H37L/L4M was more effective than H37L/L4L. IgG2 type of H37L/L4M and H37L/L4L had almost the same inhibitory activities. In short, the inhibitory activities of B-F5s against PPD-induced PBMC proliferation were as follows: H37L/L4M IgG1 > H37L/L4L IgG1 > H37L/L4M IgG2 = H37L/L4L IgG2 = mB-F5

Considering the efficacy of the *in vitro* biological activity and the smaller number of mouse amino acids, H37L/L4M IgG1 was chosen for further evaluation.

EXAMPLE 3: PRELIMINARY EVALUATION OF THE EFFECT OF hB-F5 ON PATIENTS WITH RHEUMATOID ARTHRITIS (RA).

The effect of hB-F5 (H37L/L4M IgG1) was tested on RA patients.

The conditions of the assay are as follows:

Each patient received a 10 days treatment consisting of 5 injections of 5 mg of hB-F5 (an injection every 2nd day).

The results for 3 different patients are shown in Tables II-IV below:

Patient 1 (Table II) :

Diagnosis: Rheumatoid Arthritis, Activity 2

Rheumatoid factor: 2; Stage: 2

Sex: F; Age: 65; Onset of the disease: 1965

Additional therapy: Diclophenac 150 mg/day

Table II

Clinical investigations		Before Treatment	During treatment (days)					After treatment (weeks)
			2	4	6	8	10	4
Estimation of pain in joints (0-10)		4,5	2	2	1,5	3	2,2	3,5
Morning stiffness in minutes		360	0	0	90	90	120	20
Severity of condition (1-5)	Physician	3	3	3	2,5	3	3	3
	Patient	3	3	3	3	3	3	3
Number of swollen joints		6	6	4	3	2	2	7
Number of panful joints		25	12	6	7	13	13	23
Swelling index (0-30)		8	6	4		2	3	9
Power in hand	Right	17	15	20	22	12	20	15
	Left	10	10	10	15	12	19	12
Estimation of tiredness (0-10)		7,7	4	2,3	2	2,3	3,1	3
Estimation of treatment effects	Patient		3	3	4	3	5	2
	Physician		3	3	4	3	5	2
Erythrocyte sedimentation rate		35					34	25
C-Reactive Protein		4,0					2	2,5

Patient 2 (Table III):

Diagnosis: Rheumatoid Arthritis, Activity 3

Rheumatoid factor: 2; Stage: 2

Sex: F; age: 48; Onset of the disease: 2000

Additional therapy. Diclophenac 150 mg/day

Table III

Clinical investigations		Before Treatment	During treatment (days)					After treatment (weeks)
			2	4	6	8	10	4
Estimation of pain in joints (0-10)		8,2		8,2	5	2,9	2,2	0,6
Morning stiffness in minutes		240		120	120	60	20	10
Severity of condition (1-5)	Physician	4		3	3	3	3	2
	Patient	4		4	3	3	3	2
Number of swollen joints		13		12	11	11	5	5
Number of painful joints		22		22	16	15	13	7
Swelling index (0-30)		15		14	12	11	5	5
Power in hand	Right	30		30	28	34	36	40
	Left	22		20	18	18	22	28
Estimation of tiredness (0-10)		8,7		5,1	2,2	2,2	1,1	0,7
Estimation of treatment effects	Patient			2	4	4	4/5	5
	Physician			2	2	3	4/5	5
Erythrocyte sedimentation rate		35					38	35
C-Reactive Protein		1,2					0,2	0,8

Patient 3 (Table IV):

Diagnosis: Rheumatoid Arthritis, Activity 3

Rheumatoid factor: 3; Stage: 2

Sex: F; Age: 49; Onset of the disease: 1989

Additional therapy. Diclophenac 150 mg/day

Table IV

Clinical investigations		Before treatment	During treatment (days)						After treatment (weeks)
			2	4	6	8	10	1	2
Estimation of pain in joints (0-10)		7,9	7,6	7,6	7,2	5,0	3,0	1,5	1,3
Morning stiffness in minutes		360	0	0	0	0	0	0	
Severity of condition (1-5)	Physician	4	3	3	3	3	3	2	2
	Patient	5	4	4	3		3	2	2
Number of swollen joints		10	7	7	6	5	5	5	5
Number of painful joints		30	24	24	15	11	11	10	9
Swelling index (0-30)		15	12	12	9	7	7	6	
Power in hand	Right	24	30	30	36	48	48	50	50
	Left	24	30	30	38	40	34	40	42
Estimation of tiredness (0-10)		8,5	7,2	5,2	0	0	0	0	0
Estimation of treatment effects	Patient		3	3	3		5	5	5
	Physician		3	4/3	4	4	5	5	5
Erythrocyte sedimentation rate		61		53	42	45			41
C-Reactive Protein		8				3, 7			3, 3

EXAMPLE 4 : ACTIVATION OF CD4+CD25+ Treg CELLS BY hB-F5

Isolation of T cells:

1) T regulatory cells (Tregs):

- CD25+ cells are isolated using CD25 microbeads;
- Depletion of contaminations: CD14-, CD8-, CD19-positive cells is made with CD14/CD8/CD19 DYNALbeads;
- Depletion of CD45RA+ cells is made with CD45RA mAb + anti-mouse DYNALbeads: purity:> 95% CD4+CD25+ Tregs

2) Effector cells

- CD4+T cells are isolated using CD4 microbeads
- Depletion of CD45RO+ cells is made with CD45RO+ mAb + anti-mouse DYNALbeads; purity:> 98% CD4/CD45RA+, CD25- effector T cells

3) Test system:

CD25+ Tregs from donor A are cocultured for 2 days with syngenic CD2-depleted PBMC, without additions (negative control = no activation = no suppressive activity), or in the presence of 0.5µg/ml anti-CD3 (OKT-3 = positive control = full activation of Tregs), or in the presence of 5µg/ml or 30µg/ml hB-F5.

After extensive washing of pre-cultured cells, CD25+ Tregs cells are isolated and treated by γ-radiation (3000 rad).

4) Test of suppressive activity:

Pre-cultured CD25+ Tregs cells are cocultured for 4 days with freshly isolated CD4+ effector T cells (1:1) from donor B in the presence of APC' s (CD2-depleted PBMC) from donor A (syngenic for pre-cultured T cells (no additional activation), allogeneic for effector T cells (= allogeneic mixed lymphocyte reaction). Then, cells are incubated for 16h with ^{3}H Thymidine, and proliferation of effector T cells is detected.

The results are shown in Figure 9.

Legend of Figure 9:

- negative control (no activation) = preCD25;
- 0.5µg/ml OKT-3 (positive control, full activation) = preCD25-CD3;
- 5µg/ml hB-F5 (Test-1) = preCD25-CD4;
- 30µg/ml hB-F5 (Test-2) = preCD25-CD4.

CLAIMS

1) A humanized antibody (hB-F5) derived from mouse mouse monoclonal anti-CD4 antibody B-F5, wherein said hB-F5 antibody has V domains defined by the following polypeptide sequences:

- H chain V domain:

EEQLVESGGGLVKPGGSLRLSCAASGFSFSDCRMYWLRQAPGKGLEWIGVISVKSENYGAN YAESVRGRFTISRDDSKNTVYLQMNSLKTEDTAVYYCSASYYRYDVGAWFAYWGQGTLVTV SS (SEQ ID NO: 1)

- L chain V domain:

DIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSYIYWYQQKPGQPPKLLIYLASILESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQHSRELPWTFGQGTKVEIK (SEQ ID NO: 2).

2) A fragment of a hB-F5 antibody of claim 1, wherein said fragment comprises the V domains SEQ ID NO: 1 and SEQ ID NO: 2.

3) A polynucleotide selected among :

- a polynucleotide comprising a sequence encoding the H chain V domain of SEQ ID NO:1;

- a polynucleotide comprising a sequence encoding the L chain V domain of SEQ ID NO:2.

4) A polynucleotide according to claim 3, which is selected among:

- a polynucleotide comprising the sequence SEQ ID NO: 3;

- a polynucleotide comprising the sequence SEQ ID NO: 4.

5) A therapeutic composition comprising a humanized antibody of claim 1, or a fragment of claim 2.

6) The use of a humanized antibody of claim 1, or a fragment of claim 2, for preparing an immunosuppressive composition.

7) The use of a humanized antibody of claim 1, to activate *in vitro* $CD25^{+}CD4^{+}$ regulatory T cells.