The antibodies provided by the present invention may show a markedly stronger effect on ITIM phosphorylation of CD32B and, thus, they are expected to have a stronger impact on the negative signaling loop triggered by ITIM phosphorylation of CD32B which in turn controls B cells which are involved, inter alia, in autoimmune diseases.

Specifically, antibodies of the present invention preferably increase ITIM phosphorylation of FcγRIIB of Daudi cells in comparison to Daudi cells not treated with said antibody. The increase is preferably 1.5, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold. From prior art antibodies that bind to CD32B the advantageous properties of the antibodies of the present invention could neither have been expected nor foreseen, let alone would there have been a reasonable expectation of success to provide them, in particular the CDRs or variable heavy and/or light chain as characterized herein. In addition to this improved property of the antibodies of the present invention, the antibodies described herein may also having a high specificity for human FcγRIIB and/or are non-blocking, i.e., that their binding to the Fc receptor via their variable region(s) does not interfere with the binding of immune complexes (ICs) or aggregated IgG to the cells.

[0012] Accordingly, the present invention provides an anti-FcγRIIB antibody, preferably of an IgG-type, which may comprise in its heavy chain variable region H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NOs. 29, 30 and 31 and in its light chain variable region L-CDR1, L-CDR2 and L-CDR3 shown in SEQ ID NOs. 32, 33 and 34. Such an antibody increases ITIM phosphorylation of FcγRIIB of Daudi cells about 4 to 10-fold in comparison to Daudi cells not treated with said antibody.

It is apparent from Figure 7 that prior art antibodies GB3 (see WO 2005/051999) and 2B6 (see WO 2004/016750) are not able to increase ITIM phosphorylation of FcγRIIB as can be increased by an antibody of the present invention, such as 8A6 – either as chimeric or humanized 8A6 antibody. The ability or inability, respectively, to increase ITIM phosphorylation of FcγRIIB seems thus to be dependent on the CDRs, particularly on some key residues which are present in 8A6, but not in GB3 and/or 2B6, respectively. Hence, amino acids that are only present in CDRs of 8A6 at positions that correspond to the respective positions within a CDR of 2B6 or GB3 may be regarded as “key residues”..

[0013] Visual comparison of the CDRs from 2B6, GB3 and 8A6 for key residues reveals that an antibody of the present invention may comprise in H-CDR1 the amino acid sequence shown in SEQ ID NO. 29, in H-CDR2 it comprises the amino acid sequence shown in SEQ ID NO. 30, in H-CDR3 it comprises the amino acid sequence shown in SEQ ID NO. 31, in L-CDR1 it comprises the amino acid sequence shown in SEQ ID NO. 32, in L-CDR2 it comprises the amino acid sequence shown in SEQ ID NO. 33 and in L-CDR3 it comprises the amino acid sequence shown in SEQ ID NO. 34.

[0014] The differences between the amino acid sequences of the CDRs of 8A6, GB3 and 2B6 can also be expressed as degree identity (in %-identity) that is allowed in the CDRs of an antibody of the present techniques when using the CDRs of 8A6 as reference sequences. Accordingly, an H-CDR1 of an antibody of the present invention is preferably characterized as being 60% or more, such as 70%, 80% or 90% identical to the H-CDR1 as shown in SEQ ID NO. 20.

An H-CDR2 of an antibody of the present invention is preferably characterized as being 36% or more, such as 40%, 50%, 60%, 70%, 80%, or 90% identical to the H-CDR2 as shown in SEQ ID NO. 21.

An H-CDR3 of an antibody of the present invention is preferably characterized as being 50% or more, such as 60%, 70%, 80%, or 90% identical to the H-CDR3 as shown in SEQ ID NO. 22.

An L-CDR1 of an antibody of the present invention is preferably characterized as being 64% or more, such as 70%, 80%, or 90% identical to the L-CDR1 as shown in SEQ ID NO. 23.

An L-CDR2 of an antibody of the present invention is preferably characterized as being 29% or more, such as 30%, 40%, 50%, 60%, 70%, 80%, or 90% identical to the L-CDR2 as shown in SEQ ID NO. 24.

An L-CDR3 of an antibody of the present invention is preferably characterized as being 78% or more, such as 80%, or 90% identical to the L-CDR3 as shown in SEQ ID NO. 25.

[0015] Accordingly, the present invention provides an anti-FcγRIIB antibody which comprises in its heavy chain variable region an H-CDR1 sequence which is 60% or more identical to the H-CDR1 sequence shown in SEQ ID NO. 20, an H-CDR2 sequence which is 36% or more identical to the H-CDR2 sequence shown in SEQ ID NO. 21, an H-CDR3 sequence which is 50% or more identical to the H-CDR3 sequence shown in SEQ ID NO. 22, a L-CDR1 sequence which is 64% or more identical to the L-CDR1 sequence shown in SEQ ID NO. 23, a L-CDR2 sequence which is 29% or more identical to the L-CDR2 sequence shown in SEQ ID NO. 24, and a L-CDR3 sequence which is 78% or more identical to the L-CDR3 sequence shown in SEQ ID NO. 25, wherein said antibody, used at a concentration between 5 µg/m and 50 µg/mL, increases ITIM phosphorylation of FcγRIIB of Daudi cells about 4 to 10-fold in comparison to Daudi cells not treated with said antibody.

Preferably, such an antibody still comprises in its heavy and light chain variable region CDRs the “key residues” as defined in SEQ ID NOs. 29, 30, 31 (H-CDRs) and as defined in SEQ ID NOs. 32, 33 and 34 (L-CDRs).

[0016] As used herein, the term “% identity” refers to the percentage of identical amino acid residues at the corresponding position within the sequence when comparing two amino acid sequences with an optimal sequence alignment as exemplified by the ClustalW or X

techniques as available from www.clustal.org, or equivalent techniques. For example, in case of CDR alignments, each of the CDRs (from the heavy and light chain variable region, respectively) shown in SEQ ID NOs. 20-25 serves as reference sequence for a CDR sequence of interest of a heavy or light chain variable region, respectively, e.g. H-CDR1 of SEQ ID NO. 20 is aligned with an H-CDR1 of interest. Accordingly, both sequences (reference sequence and sequence of interest) are aligned, identical amino acid residues between both sequences are identified and the total number of identical amino acids is divided by the total number of amino acids (amino acid length) of SEQ ID NO. 20, 21, 22, 23,24, or 25, respectively, dependent on whether H-CDR1, H-CDR2, H-CDR3, L-CDR1, L-CDR2, or L-CDR3 are aligned. The result of this division is a percent value, i.e. percent identity value/degree.

[0017] The H-CDR1 sequences shown in SEQ ID NOs. 14 and 20 are preferred species sequences of the H-CDR1 shown in SEQ ID NO. 29.
The H-CDR2 sequences shown in SEQ ID NOs. 15 and 21 are preferred species sequences of the H-CDR2 shown in SEQ ID NO. 30.
The H-CDR3 sequences shown in SEQ ID NOs. 16 and 22 are preferred species sequences of the H-CDR3 shown in SEQ ID NO. 31.
The L-CDR1 sequences shown in SEQ ID NOs. 17 and 23 are preferred species sequences of the L-CDR1 shown in SEQ ID NO. 32.
The L-CDR2 sequences shown in SEQ ID NOs. 18 and 24 are preferred species sequences of the L-CDR2 shown in SEQ ID NO. 33.
The L-CDR3 sequences shown in SEQ ID NOs. 19 and 25 are preferred species sequences of the L-CDR3 shown in SEQ ID NO. 34.

[0018] Accordingly, there is disclosed an anti-FcγRIIB antibody, preferably of an IgG-type, which

(a) comprises in its heavy chain variable region H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NOs. 14, 15 and 16 and in its light chain variable region L-CDR1, L-CDR2 and L-CDR3 shown in SEQ ID NOs. 17, 18 and 19; or

(b) comprises in its heavy chain variable region H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NOs. 20, 21 and 22 and in its light chain variable region L-CDR1, L-CDR2 and L-CDR3 shown in SEQ ID NOs. 23, 24 and 25,

wherein said antibody preferably increases ITIM phosphorylation of FcγRIIB of Daudi cells about 4 to 10-fold in comparison to Daudi cells not treated with said antibody.

[0019] Anti-FcγRIIB antibodies comprising in its heavy chain variable region H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NOs. 14, 15 and 16 and in its light chain variable

region L-CDR1, L-CDR2 and L-CDR3 shown in SEQ ID NOs. 17, 18 and 19, or having in its heavy chain variable region H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NOs. 20, 21 and 22 and in its light chain variable region L-CDR1, L-CDR2 and L-CDR3 shown in SEQ ID NOs. 23, 24 and 25 are preferred antibodies. These preferred antibodies preferably increase ITIM phosphorylation of FcγRIIB of Daudi cells about 4 to 10-fold in comparison to Daudi cells not treated with said antibody.

[0020] An “antibody” when used herein is a protein comprising one or more polypeptides (comprising one or more binding domains, preferably antigen binding domains) substantially or partially encoded by immunoglobulin genes or fragments of immunoglobulin genes. The term "immunoglobulin" (Ig) is used interchangeably with "antibody" herein. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. In particular, an “antibody” when used herein, is typically tetrameric glycosylated proteins composed of two light (L) chains of approximately 25 kDa each and two heavy (H) chains of approximately 50 kDa each. Two types of light chain, termed lambda and kappa, may be found in antibodies. Depending on the amino acid sequence of the constant domain of heavy chains, immunoglobulins can be assigned to five major classes: A, D, E, G, and M, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2, with IgG being preferred in the context of the present disclosure. An antibody of the present disclosure is also envisaged which has an IgE constant domain or portion thereof that is bound by the Fc epsilon receptor I. An IgM antibody consists of 5 of the basic heterotetramer unit along with an additional polypeptide called a J chain, and contains 10 antigen binding sites, while IgA antibodies comprise from 2-5 of the basic 4-chain units which can polymerize to form polyvalent assemblages in combination with the J chain. In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each light chain includes an N-terminal variable (V) domain (VL) and a constant (C) domain (CL). Each heavy chain includes an N-terminal V domain (VH), three or four C domains (CHs), and a hinge region.

The constant domains are not involved directly in binding an antibody to an antigen, but can exhibit various effector functions, such as participation of the antibody dependent cellular cytotoxicity (ADCC). If an antibody should exert ADCC, it is preferably of the IgG1 subtype, while the IgG4 subtype would not have the capability to exert ADCC.

[0021] When used herein the term "antibody" does not only refer to an immunoglobulin (or intact antibody), but also refers to a fragment thereof, and encompasses any polypeptide comprising an antigen-binding fragment or an antigen-binding domain. Preferably, the fragment such as Fab, F(ab'), $F(ab')_2$, Fv, scFv, Fd, disulfide-linked Fvs (sdFv), and other antibody fragments that retain antigen-binding function as described herein. Typically, such

specifically bind to other Fc receptors. The antibodies or fragments or derivatives thereof according to the disclosure may bind to FcγRIIB through the variable domain of the antibody. However, these antibodies may also be bound by the Fc gamma RIIB through their Fc domain.

[0026] The pairing of a VH and VL together forms a single antigen-binding site. The CH domain most proximal to VH is designated as CH1. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. The VH and VL domains consist of four regions of relatively conserved sequences called framework regions (FR1, FR2, FR3, and FR4), which form a scaffold for three regions of hypervariable sequences (complementarity determining regions, CDRs). The CDRs contain most of the residues responsible for specific interactions of the antibody with the antigen. CDRs are referred to as CDR 1, CDR2, and CDR3. Accordingly, CDR constituents on the heavy chain are referred to as H1 or H-CDR1, H2 or H-CDR2 and H3 or H-CDR3, while CDR constituents on the light chain are referred to as L1 or L-CDR1, L2 or L-CDR2, and L3 or L-CDR3.

[0027] The term "variable" refers to the portions of the immunoglobulin domains that exhibit variability in their sequence and that are involved in determining the specificity and binding affinity of a particular antibody (i.e., the "variable domain(s)"). Variability is not evenly distributed throughout the variable domains of antibodies; it is concentrated in sub-domains of each of the heavy and light chain variable regions. These sub-domains are called "complementarity determining regions" (CDRs). The terms "CDR", and its plural "CDRs", refer to a complementarity determining region (CDR) of which three make up the binding character of a light chain variable region (L1-CDRL1, L2-CDR and L3-CDR) and three make up the binding character of a heavy chain variable region (H1-CDR, H2-CDR and H3-CDR). CDRs contribute to the functional activity of an antibody molecule and are separated by amino acid sequences that comprise scaffolding or framework regions. The exact definitional CDR boundaries and lengths are subject to different classification and numbering systems. CDRs may therefore be referred to by Kabat, Chothia, contact or any other boundary definitions, including the numbering system described herein. Despite differing boundaries, each of these systems has some degree of overlap in what constitutes the so called "hypervariable regions" within the variable sequences. CDR definitions according to these systems may therefore differ in length and boundary areas with respect to the adjacent framework region. See for example Kabat, Chothia, and/or MacCallum et al., (Kabat et al., loc. cit.; Chothia et al., J. Mol. Biol, 1987, 196: 901; and MacCallum et al, J. Mol. Biol, 1996, 262: 732). However, the numbering in accordance with the so-called Kabat system is preferred.

[0028] Preferred variable regions of an antibody of the present invention are shown in SEQ ID Nos. 1, 2, 3, and 4.

[0029] The term "framework region" refers to the art-recognized portions of an antibody variable region that exist between the more divergent (i.e., hypervariable) CDRs. Such framework regions are typically referred to as frameworks 1 through 4 (FR1, FR2, FR3, and FR4) and provide a scaffold for the presentation of the six CDRs (three from the heavy chain and three from the light chain) in three dimensional space, to form an antigen-binding surface.

[0030] Antibodies (including fragments and derivatives thereof) of the present invention preferably or advantageously increase ITIM phosphorylation of FcγRIIB of Daudi cells about 4 or more-fold, such as about 4-fold or more, about 5-fold or more, about 6-fold or more, about 7-fold or more, about 8-fold or more, about 9-fold or more or about 10-fold (i.e. even nearly 10-fold) in comparison to Daudi cells not treated with said antibody.. For that comparison, both an antibody of the present invention is preferably used in an amount within the range of 5 μg/ml to 50 μg/ml, such as 10, 15, 20, or 25 μg/ml.

From the results shown in Figures 6, 7 and 8, it is apparent that either the chimeric 8A6 (ch8A6) antibody (comprising rat variable regions and a human constant region) or the humanized 8A6 antibody (hu8A6) markedly increase ITIM phosphorylation in comparison to the prior art antibody GB3. Bearing in mind that the CDRs between the chimeric and humanized 8A6 antibodies are nearly identical, while their framework regions (FRs) are different, and the potency of both antibodies to increase ITIM phosphorylation of FcγRIIBs is nearly the same (see Figure 8), it is reasonable to conclude that the CDRs are causative for the advantageous property of the antibodies of the present invention to markedly increase ITIM phosphorylation, for example, in comparison to the antibody GB3.

The skilled person is readily in a position to graft the CDRs as described herein for the antibodies of the present invention into an appropriate framework or, vice versa, graft framework regions into an antibody having the CDRs of an antibody of the present invention such that the thus-resulting antibody has the advantageous properties, in particular the property of increasing ITIM phosphorylation of CD32B as described herein.

[0031] As mentioned, antibodies of the present techniques have preferably or advantageously the property to increase ITIM phosphorylation of FcγRIIB (CD32B) of Daudi cells, for example, in comparison to the prior art antibody GB3 described in WO 2005/051999, which is characterized as having the variable region of the heavy chain shown in SEQ ID NO: 7 of WO 2005/051999 (see SEQ ID NO. 26) and the variable region of the light chain shown in SEQ ID NO: 5 of WO 2005/051999 (see SEQ ID NO. 27).

The increase in ITIM phosphorylation of FcγRIIB (CD32B) of Daudi cells effected by an antibody encompassed by the present techniques is preferably about 4-fold or more, about 5-fold or more, about 6-fold or more, about 7-fold or more, about 8-fold or more, about 9-fold or more or about 10-fold (i.e. even nearly 10-fold) in comparison to Daudi cells not treated with said antibody.

ITIM phosphorylation of CD32B (Fc gamma IIB receptor) of Daudi cells is preferably determined as follows:

$3x10^5$ Daudi cells suspended in RPMI 1640 medium supplemented with 1% FBS (fetal bovine serum) are either left untreated (control) or incubated for 25 minutes at 37°C, 5% CO_2 with an antibody mix containing anti-IgM (anti-human, mouse) and anti-mouse IgG (rabbit) wherein the antibody mix comprises 2 µg/ml α-hIgM (mAB, clone UHB) and 20 µg/ml α-mIgG. Subsequently the cells are either treated 20 minutes at 37°C, 5% CO_2 with an antibody encompassed by the present invention or with an antibody of interest as defined herein below, such as the GB3 antibody of WO 2005/051999, respectively, both antibodies are preferably applied at equal concentration, and optionally with buffer as control (w/o). Cells are harvested after incubation at 4°C, lysed and subjected to Western Blot-analysis, whereby phosphorylation is detected by an (anti-) phosphotyrosine antibody (anti-CD32B (phospho Y292) antibody). The Western Blot is optionally probed with an antibody detecting e.g. ß-Actin which serves as loading control for Western Blot-analysis. As an alternative to Daudi cells, PBMCs or Raji cells can be used. Accordingly, in all embodiments which apply Daudi cells when determining ITIM phosphorylation Daudi cells can be replaced by Raji cells or PBMCs.

[0032] The phosphotyrosine antibody is preferably coupled to a signal generating group. A signal generating group refers to a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include radiolabels such as ^{32}P, ^{35}S, or ^{125}I; fluorescent dyes (for example, Cy-3, Cy-5); chromophores, electron-dense reagents; enzymes that generate a detectable signal (e.g., as commonly used in an ELISA); or spin labels. The label or detectable moiety has or generates a measurable signal, such as a radioactive, chromogenic, or fluorescent signal, that can be used to quantify the amount of bound detectable moiety in a sample.

The signal generating group can be covalently or non-covalently bound to the phosphotyrosine antibody. A signal can be determined by way of the signal provided by the signal generating group of a phosp hotyrosine antibody. The signal can be any signal which is detectable by, for example, spectroscopic, photochemical, biochemical, immunochemical, or chemical means.

[0033] An increase in ITIM phosphorylation is determined by comparing (i) the signal generated from the signal generating group of the phosphotyrosine antibody bound to the ITIM motif of CD32B of cells that were untreated ("reference value") to (ii) the signal generated from the signal generating group of the phosphotyrosine antibody bound to the ITIM motif of CD32B of cells that were treated with an antibody encompassed by the present techniques, whereby if signal (ii) is higher than signal (i) an increase in ITIM phosphorylation of CD32B was effected by an antibody encompassed by the present techniques. For that comparison an antibody of the present invention is preferably used in an amount within the range of 5 μg/ml to 50 μg/ml, such as 10, 15, 20, or 25 μg/ml. For example, when comparing the prior art antibody GB3 or any other antibody binding to CD32B (collectively called "antibody of interest"), preferably one that binds the epitope on CD32B as described herein and/or that is non-blocking as described herein with an antibody encompassed by the present invention in order to determine the ability of an antibody of interest and an antibody encompassed by the present invention as to how many-fold each antibody may increase ITIM phosphorylation of CD32B, ITIM phosphorylation is determined as described above for the antibody of interest and an antibody encompassed by the present invention. Namely, a value for the comparison of an antibody of interest with untreated cells and a value for the comparison of an antibody encompassed by the present invention with untreated cells is obtained. These values can be compared to each other in order to determine whether an antibody encompassed by the present invention has the ability to increase ITIM phosphorylation to a higher extent, such as 4 to 10-fold (including 4, 5, 6, 7, 8, 9, 10) than an antibody of interest. For that comparison, an antibody of interest and an antibody of the present invention are preferably used in an amount within the range of 5 μg/ml to 50 μg/ml, such as 10, 15, 20, or 25 μg/ml.

[0034] The term "Fc gamma receptor IIB" is used herein interchangeably with "FcgRIIB" or "Fcgamma receptor IIB" or "Fcγ receptor IIB" or "FcγRIIB" and comprises both membranous FcγRIIB and soluble (i.e. the extracellular part of a Fcγ IIB receptor) FcγRIIB. Said term also includes variants of FcγRIIB such as FcγRIIB1 and FcγRIIB2 which differ from each other in a 19 amino acid sequence insertion in the cytoplasmic domain of FcγRIIB1. Another variant encompassed by said term is FcγRIIB3 which is identical to FcγRIIB2, but lacks information for the putative signal peptidase cleavage site.
Sometimes, FcγRIIB is also referred to herein as „CD32B". Thus this term as well as the other terms used to designate Fc gamma receptor IIB as described above, can be interchangeably used with the term "CD32B". Fc gamma receptor IIB belongs to the immunoglobulin superfamily of proteins and is found on many hematopoietic lineages. As its name indicates, Fc receptor IIB recognizes and binds to the Fc (fragment, crystallizable) part

[0040] As regards the heavy chain variable region of an antibody of the present invention, it is preferred that the heavy chain variable region of an antibody of the present invention comprises the amino acid sequence shown in SEQ ID NO. 3, with at least one of the mutations selected from the group consisting of amino acid Q at position 1 being replaced by E, amino acid V at position 11 being replaced by L, amino acid G at position 42 being replaced by K, amino acid S at position 50 being replaced by V, amino acid Y at position 53 being replaced by S, amino acid K at position 58 being replaced by T, amino acid G at position 61 being replaced by A, amino acid S at position 75 being replaced by T, amino acid K at position 76 being replaced by R, amino acid N at position 77 being replaced by S, and amino acid T at position 78 being replaced by N. Such an antibody is preferably characterized as comprising the heavy chain constant region the amino acid sequence shown in SEQ ID NO. 6 and/or the light chain constant region the amino acid sequence shown in SEQ ID NO. 7.

[0041] An antibody of the present invention is preferably characterized as comprising the heavy chain constant region the amino acid sequence shown in SEQ ID NO. 6 and/or the light chain constant region the amino acid sequence shown in SEQ ID NO. 7.

[0042] In a preferred antibody of the invention, the constant region of the heavy chain contains an alanine residue at position 297 (N297A) according to the EU protein numbering as described by Edelman *et al.* 1969 (corresponds to the numbering of the sequence which is represented by SEQ ID NO.6 as shown in Figure 2). Antibodies with a heavy chain containing an alanine (Ala, A) residue at position 297 (N297A) are designated herein with the suffix "_N297A", while antibodies having an asparagine (Asn, N) residue at said position are "wildtype" and are thus designated herein with the suffix "(wt)". As can be seen in Figure 2, the variable region of the heavy chain of the humanized antibody 8A6 wild type ends with an amino acid residue "S" at position 113 according to the EU protein numbering. The constant region of said antibody starts at position 118. The resulting apparent gap of 4 amino acid residues is caused by the switch to the EU protein numbering system for the constant region and does not mean that any amino acid residues are missing.

The antibodies according to the invention having an alanine residue at position 297 of the amino acid sequence represented by SEQ ID NO.6 do have a reduced or no antibody dependent cellular cytotoxicity due to a reduced or non-existent binding of the Fc part of the antibody to Fc receptors. The amino acid sequence of such a N297A constant region is shown in SEQ ID NO. 28. Accordingly, antibodies of the present invention may contain as constant region the amino acid sequence shown in SEQ ID NO. 28. Such antibodies lack glycosylation at position 297 according to the EU protein numbering. Thus, the present invention may encompass antibodies that lack glycosylation at position 297 according to the EU protein numbering of the heavy chain constant region, but also encompasses antibodies that

[0058] The antibody or fragment or derivative thereof according to the present invention can advantageously be used in a pharmaceutical composition. Such pharmaceutical composition can be applied for the treatment or prophylaxis of diseases or disorders, preferably autoimmune diseases.

FcγRIIB-specific antibodies according to the invention may inhibit the immune response in B-cells, dendritc cells and activated granulocytes (e.g., macrophages) which leads to a reduced production of immune stimulatory mediators and to a reduction in antibody production as well as antigen presentation (e. g., on dendritic cells and macrophages leading to a decrease in T-cell recruitment). Taken together the feedback loop of antibody production and restimulation of the immune system is inhibited.

Importantly, the binding of the anti-FcγRII antibody of the invention to the receptor via its variable regions does not interfere with Fc-fragment binding of ICs or antibodies to the receptor, and in contrast to known blocking antibodies the normal function of the Fc-receptor is maintained.

[0059] Therefore, in another aspect the present disclosure relates to a pharmaceutical composition comprising as an active ingredient an antibody or fragment or derivative thereof according to the invention. Said pharmaceutical composition may comprise at least one pharmaceutically acceptable carrier or adjuvant or excipient.

Antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as listed in a generally recognized pharmacopeia for use in animals, and more particular in humans.

[0060] The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

The compositions of the invention can be formulated as neutral or salt forms.

Pharmaceutically acceptable salts include, but are not limited to those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0061] The above-mentioned pharmaceutical composition can be used for the treatment or prophylaxis or diagnosis of any disease or disorder, preferably of autoimmune diseases, and most preferably of autoimmune diseases characterized by the production of auto-antibodies.

As used herein, the term "treating" and "treatment" refers to administering to a subject a therapeutically effective amount of a pharmaceutical composition according to the invention. A “therapeutically effective amount” refers to an amount of the pharmaceutical composition or the antibody which is sufficient to treat or ameliorate a disease or disorder, to delay the onset of a disease or to provide any therapeutical benefit in the treatment or management of a disease.
As used herein, the term “prophylaxis” refers to the use of an agent for the prevention of the onset of a disease or disorder. A “prophylacticly effective amount” defines an amount of the active component or pharmaceutical agent sufficient to prevent the onset or recurrence of a disease.
As used herein, the terms "disorder" and "disease" are used interchangeably to refer to a condition in a subject. In particular, the term "autoimmune disease" is used interchangeably with the term "autoimmune disorder" to refer to a condition in a subject characterized by cellular, tissue and/or organ injury caused by an immunologic reaction of the subject to its own cells, tissues and/or organs.

[0064] Moreover, antibodies of the invention can be used for diagnostic purposes to detect, diagnose, or monitor diseases, or disorders, in particular autoimmune diseases. Antibodies or fragments or derivatives thereof according to the invention can be used to assay FcγRIIB levels in a biological sample using classical immunohistological methods as described herein or as known to those of skill in the art (e.g., see Jalkanen et al., 1985, J. Cell. Biol. 101: 976-985; Jalkanen et al., 1987, J. Cell. Biol. 105: 3087-3096). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA).
Therefore, the present disclosure further relates to a diagnostic composition comprising an antibody of the invention.
As used herein, the term “diagnostic” refers to any use of the inventive antibody for diagnosing the presence of a FcγRIIB related auto-immune disease, for example the determination of the amount or degree of the surface expression of endogenous FcγRIIB on cells of an individual or patient. It also refers to the determination of the ratio of activatory FcγRIIs to inhibitory FcγRIIs, e.g. the ratio of expressed FcγRIIA to FcγRIIB.

[0065] In a preferred technique the diagnostic composition as described above is for the detection and diagnosis of any disease or disorder, especially autoimmune diseases characterized by the production of auto-antibodies. Exemplary autoimmune diseases include Immune Thrombocytopenia, Systemic Lupus Erythematosus, Pernicious Anemia, Addison’s disease, Diabetis type 1, Rheumatoid Arthritis, Sjogren’s syndrome,

Dermato-myositis, Multiple Sclerosis, Myasthenia gravis, Reiter's syndrome, Graves' disease, Pemphigus vulgaris and bullosus, autoimmune Hepatitis, ulcerative Colitis, cold agglutinin disease, Autoimmune peripheral neuropathy, but are not limited to these.

[0066] In a preferred embodiment the antibodies of the invention are used for the diagnosis of an autoimmune disease in a human. For example, the antibodies according to the invention can be used to determine the surface expression of FcγRIIB on the cells of an individual suffering from an auto-immune disease Preferably the expression of FcγRIIB on B cells or Plasma cells is detected using the antibody in a FACS analysis. For this the antibody can be modified by a marker reagent, a fluorescent maker or any other marker known to the person skilled in the art to be able to detect using standard procedures. The antibody according to the invention can also be used as a diagnostic tool in combination with antibodies specific for human FcγRIIA known in the state of the art. Thus the expression of FcγRIIB and FcγRIIB on the cells of an individual suffering from an auto-immune disease or an inflammatory disease can be determined and a ratio can be calculated that is a marker for the disease state or progression of the disease or as a marker for diagnosing the disease.

[0067] The present disclosure further provides a diagnostic kit for the detection of autoimmune diseases comprising an antibody or fragment or derivative thereof according to the invention, and optionally marker reagents, carrier reagents and/or suitable and required receptacles.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1: Surface Plasmon Resonance analysis of humanized 8A6 according to SEQ. ID. No. 3 and 4 in either wildtype or N297A format, ch8A6_N297A (according to SEQ. ID. NO. 14 and 15 and chGB3_N297A.

FIGURE 2: Sequences of hu8A6_wt and hu8A6_N297A depicting position of N to A amino acid exchange in N297A format.

FIGURE 3: Non-blocking characteristic of ch8A6_N297A. Raji cells were incubated with a set amount of aggregated human IgG and varying amounts of ch8A6_N297A, chGB3_N297A or blocking antibodies 2B6 or R&D Ab. The exemplary antibodies are non-blocking.

FIGURE 4: Binding from 15 µg/ml to 0.005 µg/ml of Protein A purified antibody (hu8A6_VL + hu8A6_VH and ch8A6_N297A) to native FcγRIIB expressed on Raji cells. Humanized 8A6 variants bind with high avidity to FcγRIIB expressed on Raji cells.

FIGURE 5: Binding of 15 µg/ml antibody (hu8A6_VH + hu8A6_VL and ch8A6_N297A) to native FcγRIIA expressed on K562 cells. Exemplary antibodies do not bind to FcγRIIA on K-562.

FIGURE 6a: ITIM-Phosphorylation increased by chimeric 8A6 (ch8A6_N297A) in PBMC from healthy donor. PBMC from healthy donor were isolated using Ficoll separation and subsequently left untreated or incubated for 25 minutes with an antibody mix containing anti-IgM (anti-human, mouse) and anti-mouse IgG (rabbit). Subsequently the cells were either treated 20 minutes with 5µg/mL ch8A6_N297A or buffer as control (w/o). Cells were harvested after incubation and lysed according to protocol. Lysates were subjected to WB-analysis. ß-Actin = loading control.

FIGURE 6b: Control experiment for ITIM-Phosphorylation. Daudi cells were left untreated or treated for 25 minutes with an isotype control antibody, polyclonal anti-human anti-IgM (polycl. anti-hIgM), monoclonal anti human IgM (anti-hIgM), anti-hIgM + 5µg/mL ch8A6_N297A, anti-mouse IgG from rabbit (α-mouseIgG), α-mouse IgG+5µg/mL ch8A6_N297A, mix of anti-hIgM and α-mouseIgG (Ab mix) or Ab mix + 5µg/mL ch8A6_N297A). ß-Actin = loading control.

FIGURE 6c: Antibodies of the present invention may enhance ITIM phosphorylation in the presence or absence of crosslinking/colligation of BCR and Fcgamma RIIB in primary PBMCs.

FIGURE 7: Comparison of ch8A6_N297A with antibody from the state of the art (chGB3_N297A). Human Daudi cells were incubated for 25 minutes with an antibody mix containing anti-IgM (anti-human, mouse) and anti-mouse IgG (rabbit) or left untreated. Subsequently the cells were either treated 20 minutes with varying amounts of chGB3_N297A or ch8A6_N297A or buffer as control (w/o). Cells were harvested after incubation and lysed according to protocol. Lysates were subjected to WB-analysis. ß-Actin = loading control.

FIGURE 8: Comparison of the effect of the humanized variant hu8A6_N297A and ch8A6_N297A and chGB3_N297A on ITIM phosphorylation in primary PBMCs. After crosslinking of BCR and FcγRIIB by the antibody mix, the different antibodies were added at 5 µg/ml and Western Blot analysis for ITIM phosphorylation was conducted. ß-Actin = loading control.

FIGURE 9: Co-Immunoprecipitation of phosphorylated SHIP-1 with FcγRIIB ITIM. After stimulation of Daudi cells with the antibody mix and either ch8A6_N297A, blocking anti-FcγRIIB antibody 2B6 or chGB3_N297A (5µg /mL), FcγRIIB was precipitated from cell lysates and Western Blot analysis was performed for the phosphatase SHIP-1. Anti-CD32 using pan anti-CD32 antibody (AF1330) = loading control.

FIGURE 10: Comparison of humanized 8A6 variants (hu8A6-VH10+VL2/VH10+VL6/ VH10+VL7/VH12+VL2/VH12+VL6/VH12+VL7) to ch8A6_N297A. Daudi cells were left incubated for 25 minutes with buffer (untreated) or a mix (antibody mix) containing anti-IgM (anti-human, mouse) and anti-mouse IgG (rabbit). Subsequently the cells were either treated 20 minutes with 0.25µg/mL of ch8A6_N297A or humanized 8A6 variants (hu8A6-VH10+VL2/VH10+VL6/ VH10+VL7/VH12+VL2/VH12+VL6/VH12+VL7). Cells were harvested after incubation and lysed according to protocol. Lysates were subjected to WB-analysis. ß-Actin = loading control.

FIGURE 11: Comparison of humanized 8A6 variants (hu8A6-VH10+VL2/VH10+VL6/ VH10+VL7/VH12+VL2/VH12+VL6/VH12+VL7) to ch8A6_N297A, blocking anti-FcγRIIB and chGB3_N297A. Daudi cells were left incubated for 25 minutes with buffer (untreated) or a mix (antibody mix) containing anti-IgM (anti-human, mouse) and anti-mouse IgG (rabbit).

Subsequently the cells were either treated 20 minutes with 0.25µg/mL of ch8A6_N297A, humanized 8A6 variants (hu8A6-VH10+VL2/VH10+VL6/ VH10+VL7/VH12+VL2/VH12+VL6/VH12+VL7),, blocking anti-FcγRIIB antibody 2B6 or chGB3_N297A. Cells were harvested after incubation, lysed according to protocol and analyzed in a Western Blot assay. Lysates were subjected to WB-analysis. ß-Actin = loading control.

FIGURE 12: Experimental setup for SLE-PBL mouse model. PBL from human SLE patients are transfered into immuno-compromised mice. PBL cells are engrafted and mice are subsequently treated with control (PBS) or anti-FcγRIIB ch8A6_N297A antibody according to an example of the invention.

FIGURE 13: Total human IgG level [µg/mL] in mice grafted with PBL from human donors suffering from SLE. Depicted are mice treated with control (#2, PBS) or ch8A6_N297A (#3 and #4).

FIGURE 14: Reduction in disease specific human anti-DNA IgG in ch8A6_N297A treated mice starting in week 4 post SLE-PBL transfer/grafting using PBL from human donors suffering from SLE. Depicted are anti-DNA IgG titers in two different mice, #3 and #4 (treated with ch8A6_N297A), #2 shows PBS control

with ch8A6 did not show an increased *FcγRIIB*-ITIM-phosphorylation (FIG. 6b). The antibodies of the invention may thus show a markedly effect on the ITIM-phosphorylation of human cells with crosslinked BCR and membrane-bound (endogenously expressed) FcγRIIB and not on unstimulated cells, i.e. cells without crosslinked BCR and membrane-bound FcγRIIB. During an auto-immune disease, BCR and membrane-bound FcγRIIB will be crosslinked by auto-antigens or immune complexes (ICs). The inventive antibodies are able to inhibit pathogenic autoreactive B cells in an auto-immune disease by increasing *FcγRIIB*-ITIM-phosphorylation. However, antibodies of the present invention may also be able to increase ITIM-phosphorylation without crosslinked BCR (FIG. 6c).

Comparison of the effects of ch8A6 with antibody from the state of the art (chGB3_N297A)

Comparison of the effect of clone ch8A6_N297A and clone chGB3_N297A on ITIM phosphorylation. Human Daudi cells were treated with an antibody mix and, subsequently, ch8A6_N297A, chGB3_N297A or 2B6 as described above. The antibody chGB3_N297A like ch8A6_N297A is a non-blocking anti-FcγRIIB antibody and recognizes a similar epitope.

Addition of ch8A6_N297A to the antibody-mix treated cells showed an increase of FcγRIIB-ITIM phosphorylation already at a concentrations of 0.05 µg/ml. Though increasing concentrations of the chGB3_N297A showed a dose-dependent stimulation of phosphorylation of the inhibitory motif, surprisingly this antibody clone was not able to reach phosphorylation levels comparable to 8A6. Densitometric quantitation of the X-ray film with the software "ImageJ" calculated values of a maximum of 2.8-fold phospho-signals, whereas hu8A6_N297A lead to a 9.8-fold increase compared to untreated cells (FIG. 7). Thus the inventive antibodies clearly and surprisingly show an increased *FcγRIIB*-ITIM-phosphorylation in comparison to antibodies in the state of the art.

Comparison of the effect of the humanized variant hu8A6, chimeric 8A6_N297A and chGB3_N297A on ITIM phosphorylation in primary PBMC

Antibody chGB3_N297A, ch8A6_N297A and humanized 8A6 were compared in their influence on *FcγRIIB*-ITIM phosphorylation of primary human PBMC. After crosslinking of BCR and FcγRIIB by the antibody mix, the different antibodies were added at 5 µg/ml and Western Blot analysis for ITIM phosphorylation was conducted. Again the inventive antibodies surprisingly have a markedly increased effect on *FcγRIIB*-ITIM-phosphorylation compared to the antibody in the state of the art (FIG. 8).

Treatment of mice was started 2 weeks following injection of cells after grafting of human SLE-patient PBL in mice was verified by the presence of human immunoglobulin M or G. Mice were treated with 200µl buffer (PBS) or 20µg antibody (ch8A6_N297A) in 200µl PBS intraperitoneally twice weekly for 4 weeks. Mice were weighed and bled to obtain serum once weekly. Serum samples were frozen at -80°C until further use (FIG. 12).

ELISA

Serum samples were analyzed by ELISA for the presence of total human IgG, IgM and anti-DNA IgM and IgG.

For quantification of total serum IgM and IgG in serum samples, the Bethyl Human IgM ELISA Quantitation Kit and the Human IgG ELISA Quantitation Kit (Biomol) were used according to the manufacturer's instructions. OD was measured with VersaMax tuneable microplate reader (Molecular Devices) at 450 and 650 nm.

For the detection of anti-DNA antibodies, ELISA plates were coated with10 µg/mL methylated BSA (Sigma) in PBS for 2 h at room temperature. After washing, the plates were coated with 50 µg/mL calf thymus DNA (Sigma) in PBS at 4 °C overnight. Blocking of unspecific binding was performed with PBS/0.1% Gelatin/3% BSA/1mM EDTA for 2 h at room temperature. Sera were diluted 1:100 in the blocking solution and incubated for 1 h at room temperature. As a detection antibody, the HRP-conjugated antibody of the human IgM Quantitation Kit (Bethyl) was used and diluted 1:10,000 in blocking solution followed by incubation for 1 h at room temperature. PBS was used for all washing steps. For detection, TMB Solution was added and the reaction was stopped with 6% orthophosphoric acid.

SLE PBL Model, total human serum immunoglobulin

Total human IgG levels [µg/mL] were analyzed in mice grafted with PBL from human donors suffering from SLE. No significant difference in total human IgG between PBS or anti-FcγRIIB was detected. The antibody according to the invention may not significantly influence total human IgG (FIG. 13).

SLE PBL Model, influence on anti-DNA antibodies (disease specific IgG)

A markedly reduction in disease specific human anti-DNA IgG in anti-FcγRIIB mice starting in week 4 post

SLE-PBL transfer/grafting was observed. The inventive antibodies specifically reduce the amount of disease relevant anti-DNA antibodies (FIG. 14).

CLAIMS

1. An anti-FcγRIIB antibody which comprises

(i) an H-CDR1 sequence which is 60% or more identical to the H-CDR1 sequence shown in SEQ ID NO. 20,

(ii) an H-CDR2 sequence which is 36% or more identical to the H-CDR2 sequence shown in SEQ ID NO. 21,

(iii) an H-CDR3 sequence which is 50% or more identical to the H-CDR3 sequence shown in SEQ ID NO. 22,

(iv) a L-CDR1 sequence which is 64% or more identical to the L-CDR1 sequence shown in SEQ ID NO. 23,

(v) a L-CDR2 sequence which is 29% or more identical to the L-CDR2 sequence shown in SEQ ID NO. 24, and

(vi) a L-CDR3 sequence which is 78% or more identical to the L-CDR3 sequence shown in SEQ ID NO. 25,

wherein said antibody, used at a concentration between 5 µg/ml and 50 µg/ml, increases ITIM phosphorylation of FcγRIIB of Daudi cells about 4 to 10-fold in comparison to Daudi cells not treated with said antibody.

2. The antibody of claim 1, which comprises in its heavy chain variable region H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NOs. 29, 30 and 31 and in its light chain variable region L-CDR1, L-CDR2 and L-CDR3 shown in SEQ ID NOs. 32, 33 and 34, wherein said antibody increases ITIM phosphorylation of FcγRIIB of Daudi cells about 4 to 10-fold in comparison to Daudi cells not treated with said antibody.

3. The antibody of any one of the preceding claims, which

(a) comprises in its heavy chain variable region H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NOs. 14, 15 and 16 and in its light chain variable region L-CDR1, L-CDR2 and L-CDR3 shown in SEQ ID NOs. 17, 18 and 19; or

(b) comprises in its heavy chain variable region H-CDR1, H-CDR2 and H-CDR3 as shown in SEQ ID NOs. 20, 21 and 22 and in its light chain variable region L-CDR1, L-CDR2 and L-CDR3 shown in SEQ ID NOs. 23, 24 and 25,

wherein said antibody increases ITIM phosphorylation of FcγRIIB of Daudi cells about 4 to 10-fold in comparison to Daudi cells not treated with said antibody.

4. The antibody of any one of the preceding claims which is chimeric or humanized.

5. The antibody of any one of the preceding claims, wherein the heavy chain variable region comprises the amino acid sequence shown in SEQ ID NO. 3, with at least one of the mutations selected from the group consisting of amino acid Q at position 1 being replaced by E, amino acid V at position 11 being replaced by L, amino acid G at position 42 being replaced by K, amino acid S at position 50 being replaced by V, amino acid Y at position 53 being replaced by S, amino acid K at position 58 being replaced by T, amino acid G at position 61 being replaced by A, amino acid S at position 75 being replaced by T, amino acid K at position 76 being replaced by R, amino acid N at position 77 being replaced by S, and amino acid T at position 78 being replaced by N, and/or wherein the light chain variable region comprises the amino acid sequence shown in SEQ ID NO. 4, with at least one of the mutations selected from the group consisting of amino acid Q at position 1 being replaced by N, amino acid S at position 28 being replaced by N, amino acid S at position 31 being replaced by T, amino acid V at position 33 being replaced by L, amino acid D at position 34 being replaced by A, amino acid Y at position 49 being replaced by F, amino acid T at position 53 being replaced by N, amino acid Y at position 55 being replaced by A, amino acid L at position 89 being replaced by Q, and amino acid N at position 93 being replaced by Y.

6. The antibody of any one of the preceding claims, which comprises the heavy chain variable region shown in SEQ ID NO. 1 or 3; and/or the light chain variable region shown in SEQ ID NO. 2 or 4.

7. The antibody of any one of the preceding claims, which specifically binds to amino acids No. 20-40 of human FcγRIIB according to SEQ ID NO. 5.

8. The antibody of any one of the preceding claims, which *in vitro* binds to human FcγRIIb with an affinity having an off- rate constant of at least 4.9 x $10^{-4}s^{-1}$, as determined by surface plasmon resonance.

9. The antibody according to any one of preceding claims, which comprises in its heavy chain constant region the amino acid sequence shown in SEQ ID NO. 6; and/or in its light chain constant region the amino acid sequence shown in SEQ ID NO. 7.

10. An anti-FcγRIIB antibody which comprises the heavy chain variable region shown in SEQ ID NO. 1 or 3 and the light chain variable region shown in SEQ ID NO. 2 or 4; and which optionally comprises in its heavy chain constant region the amino acid

sequence shown in SEQ ID NO. 6; and in its light chain constant region the amino acid sequence shown in SEQ ID NO. 7

11. A nucleic acid sequence encoding the antibody of any one of claims 1 to 10.

12. A nucleic acid vector comprising at least one of the nucleic acid sequences of claim 11 inserted into a vector molecule.

13. A host cell transfected with a nucleic acid vector according to claim 12.

14. Pharmaceutical composition comprising as an active ingredient an antibody of any one of claims 1 to 10.

15. An antibody of any one of claims 1 to 10 for use in a method of treatment or prophylaxis of an autoimmune disease, said autoimmune disease being characterized by the production of auto-antibodies; and/or for use in a method of treatment or prophylaxis of Immune Thrombocytopenia, Systemic Lupus Erythematosus, Pernicious Anemia, Addison's disease, Diabetis type 1, Rheumatoid Arthritis, Sjogren's syndrome, Dermato-myositis, Multiple Sclerosis, Myasthenia gravis, Reiter's syndrome, Graves disease, Pemphigus vulgaris and bullosus, autoimmune Hepatitis, ulcerative Colitis, cold agglutinin disease, and Autoimmune peripheral neuropathy.

16. A method for the production of an antibody of any one of claims 1 to 10, comprising culturing the host cell of claim 13 under conditions which allow expression of the nucleic acid sequence comprised by the nucleic acid vector of claim 12 and recovering the thus produced antibody.