

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
27 July 2006 (27.07.2006)

PCT

(10) International Publication Number
WO 2006/077152 A2

(51) International Patent Classification:

C07K 14/74 (2006.01) **C12N 15/62** (2006.01)

C07K 16/46 (2006.01) **A61K 38/17** (2006.01)

(21) International Application Number:

PCT/EP2006/000560

(22) International Filing Date: 23 January 2006 (23.01.2006)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/645,992 21 January 2005 (21.01.2005) US

(71) Applicant and

(72) Inventor: **UTKU, Nalân** [DE/DE]; Pfalzburger Str. 81,
10719 Berlin (DE).

(74) Agent: **STEINECKE, Peter**; MÜLLER FOTTNER
STEINECKE, Rechtsanwälte Patentanwälte, P.O. Box 31
01 40, 80102 Munich (DE).

(81) Designated States (*unless otherwise indicated, for every
kind of national protection available*): AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV,
LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI,
NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG,
SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US,
UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every
kind of regional protection available*): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT,
RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished
upon receipt of that report*

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: HLA FUSION MOLECULES AND USES THEREOF

(57) Abstract: The present invention provides a fusion molecule comprising a first domain and a second domain, wherein said first domain comprises the HLA-DR alpha 2 domain or a fragment thereof. Said fusion molecule is capable of modulating the T-cell mediated immune response. Furthermore, pharmaceutical compositions comprising said fusion molecules for the treatment of various diseases such as graft versus host disease, autoimmune diseases, allergic diseases, infectious diseases and tumors in a subject are described.



WO 2006/077152 A2

Field of the invention

The present invention generally relates to the technical field of immunology. More specifically, the present invention relates to a fusion molecule derived from MHC-class II proteins, especially from the membrane proximal domain of the HLA-DR alpha chain (HLA-DR alpha 2 protein), wherein said fusion molecule is involved in the signal transduction of T-cell activation and/or proliferation. In particular, the fusion molecule of the present invention provides immunomodulatory signals which in turn are involved in cytokine expression and/or secretion of activated T-cells. Furthermore, the present invention relates to compositions comprising said fusion molecule and to methods of modulating MHC II mediated immune responses, and treating immune response related diseases.

Background of the invention

T-cell activation is a serial process involving multiple signaling pathways and sequential changes in gene expression resulting in differentiation of T-cells into distinct subpopulations, i.e. Th1 and Th2, which are distinguishable by their pattern of cytokine production and characterize the mode of cellular immune response. The T-cell response is initiated by the interaction of the antigen-specific T-cell receptor (TCR) with peptides presented by major histocompatibility complex (MHC) molecules on the surface of antigen presenting cells (APCs). Additional signals are provided by a network of receptor-ligand interactions mediated by a number of membrane proteins such as CD28/CTLA4 and B7, CD40/CD40L, LFA-1 and ICAM-1 (Lenschow, *Science* 257 (1992), 789-792; Linsley, *Annu. Rev. Immunol.* 11 (1993), 191-212; Xu, *Immunity* 1 (1994), 423-431; Bachmann, *Immunity* 7 (1997), 549-557; Schwartz, *Cell* 71 (1992), 1065-1068) collectively called costimulatory signals (Perez, *Immunity* 6 (1997), 411). These membrane proteins can alter T-cell activation in distinct ways (Bachmann, *Immunity* 7 (1997), 549-557) and regulate the immune response by the integration of positive and negative signals provided by these molecules (Bluestone, *Immunity* 2 (1995), 555-559; Perez, *Immunity* 6 (1997), 411). Many of the agents which are effective in modulating the cellular immune response either interfere with the T-cell receptor (Cosimi, *Transplantation* 32 (1981), 535-539) block costimulatory signaling (Larsen, *Nature* 381 (1996), 434-438; Blazar, *J. Immunol.* 157 (1996), 3250-3259; Kirk, *Proc. Natl. Acad. Sci.*

USA 94 (1997), 8789-8794; Linsley, Science 257 (1992), 792-95; Turka, Proc. Natl. Acad. Sci. USA 89 (1992), 11102-11105) or inhibit intracellular activation signals downstream from these primary cell membrane triggers (Schreiber and Crabtree, Immunology Today 13 (1992), 136-42). Therapeutic prevention of T-cell activation in organ transplantation and autoimmune diseases presently relies on panimmunosuppressive drugs interfering with downstream intracellular events. Specific modulation of the immune response remains a longstanding goal in immunological research.

In view of the need of therapeutic means for the treatment of diseases related to immune responses of the human body, the technical problem of the present invention is to provide means and methods for modulation of the immune response in a subject. The solution to said technical problem is achieved by providing the embodiments characterized in the claims, and described further below.

Summary of the invention

The present invention is directed to a fusion molecule comprising a first domain comprising the HLA-DR alpha 2 domain or at least a fragment thereof and a second, preferably functional domain. In particular, said second domain of the fusion molecule of the present invention comprises at least a fragment of an immunoglobulin or derivative thereof. Furthermore, the invention is directed to a polynucleotide encoding the fusion molecule of the invention, a vector comprising said polynucleotide and to a host cell comprising said vector for manufacturing said fusion molecule. The present invention further relates to a pharmaceutical composition comprising the mentioned fusion molecule, which is used in cell or organ transplantation and for the treatment of autoimmune, allergic or infectious diseases, or for the treatment of tumors. Furthermore, the present invention is directed to a diagnostic agent, comprising said fusion molecule.

Brief description of the drawings

Fig. 1A: Complete HLA-DR alpha chain protein sequence in which the extracellular alpha 2 domain which was used for generating the HLA-DR-V5 fusion protein is underlined.

- Fig. 1B:** Purified HLA-DR alpha 2-V5 fusion protein was detected by silver staining and showed the expected size of the fusion protein at 7,5 kDa.
- Fig. 1C:** Purified HLA-DR alpha 2-V5 fusion protein was detected by Western blot analysis and showed the expected size of the fusion protein at 7,5 kDa.
- Fig. 1D:** Complete HLA-DR alpha chain protein sequence in which the domain used for generating the HLA-DR alpha 2-Fc fusion protein is underlined.
- Fig. 1E:** COS7 cells were transiently transfected with an expression vector for HLA-DR-Fc fusion protein using Eugene6 (Roche). One day after transfection medium was exchanged to OptiMEM I. After an additional 96 h HLA-DR-Fc fusion protein was purified from supernatant by Sepharose A Column. 1 mg/lane was loaded on denaturing SDS acrylamid gels. Gels were stained with silver to detect protein. Bands revealed the expected size of 45 kDa protein.
- Fig. 1F:** Western blot analysis of HLA-DR-Fc fusion protein. SDS acrylamid gels were blotted to nitrocellulose to detect protein using anti-human Fc antibody coupled to alkaline phosphatase (AP). Bands were detected using BCIP/NBT and revealed the expected size of 45 kDa protein.
- Fig. 1G:** IFN- γ and IL-10 levels in the supernatant were measured by quantitative sandwich ELISA which revealed a significant inhibition of interferon gamma expression, whereas no inhibition for IL-10 expression was observed as soluble HLA-DR alpha 2 protein inhibited the interferon gamma expression by 50% at a concentration of 100 μ g/ml, compared with the controls. The results show the summary of three independent experiments. For ELISA cytokine analysis, PBMC of human healthy donors were isolated according to the Ficoll-Paque density centrifugation protocol. 5×10^4 PBMC/well were incubated with 1 μ g/ml PHA (Sigma) at 5% CO₂, 37°C for 48 h in presence of HLA-DR-Fc (1 = 50 μ g/ml; 2 = 100 μ g/ml) and Fc control (1 = 50 μ g/ml; 2 =

100 µg/ml), respectively, in a total volume of 100 µl/well. Samples were run in triplicates on 96well-microtiter-plates. The quantitation of IFN-γ and IL-10 was measured in supernatants of PHA-stimulated cells. Cytokine level was determined using the Cytoscreen® ELISA Kit (Biosource).

Fig. 2: Intracellular IFN-γ levels in splenocytes of Balb/C mice (10-14 weeks old), treated either with HLA-DR alpha 2-Fc (200 µg in PBS) or human Fc (50 µg in PBS) following 50 µg LPS induction, were measured on FACS. The results of the human Fc treated control group was set to 100 %. Splenocytes of the HLA-DR alpha 2-Fc treated mice revealed a significant inhibition of intracellular IFN-γ expression of about 40 %.

Detailed description of the invention

The present invention relates to a fusion molecule comprising a first domain and a second domain, wherein said first domain comprises the HLA-DR alpha 2 domain or a fragment thereof. Said fusion molecule is capable of modulating the T-cell mediated immune response.

The present invention is based on investigations that the alpha 2 domain of the HLA-DR alpha molecule interacts with its natural receptor located on activated lymphocytes, especially T-cells. In particular, it could be shown in accordance with the present invention the inhibition of activated T-cell IFN-γ production by soluble HLA-DR alpha 2 fusion protein.

Without intending to be bound by theory, it is believed that fusion molecules derived from HLA-DR alpha 2 and described herein are capable of modulating the signaling of MHC class II restricted T-cells in cell-mediated immunity, for example by interfering with the interaction with its natural receptor.

The term "fusion molecule interfering with the interaction of HLA-DR alpha 2 with its corresponding receptor" means in accordance with the present invention an agent capable of inhibiting and/or modulating the interaction of HLA-DR alpha 2 with its corresponding receptor. Since the interaction of HLA-DR alpha 2 with its receptor(s) interferes with events

which are valuable in course of immune responses, such inhibitor should also be capable of modulating immune responses.

In accordance with the present invention, said fusion molecule preferably interacts with its receptor(s), for example by specific binding. "Specific binding" means "specifically interacting with", whereby said interaction may be, *inter alia*, covalently, non-covalently and/or hydrophobic. Said fusion molecules include molecules which bind to, interfere with and/or occupy relevant sites on said receptor. Examples of such molecules include (poly-) peptides or peptide-like molecules.

The terms "treatment", "treating" and the like are used herein to generally mean obtaining a desired pharmacological and/or physiological effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of partially or completely curing a disease and/or adverse effect attributed to the disease. The term "treatment" as used herein covers any treatment of a disease in a mammal, particularly a human, and includes either preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it, inhibiting the disease, i.e. arresting its development or relieving the disease, i.e. causing regression of the disease.

Furthermore, the term "subject" as employed herein relates to mammals in need of amelioration, treatment and/or prevention of immunological diseases as disclosed herein. As used herein, the term "mammal" means any member of the higher vertebrate animals included in the class Mammalia, as defined in Webster's Medical Desk Dictionary 407 (1986), and includes but is not limited to humans, other primates, pigs, dogs, and rodents (such as immune-suppressed mice). In the preferred embodiment of this invention, the mammal is a human.

Thus, the present invention relates to a fusion molecule comprising a first domain comprising the HLA-DR alpha 2 domain or at least a fragment thereof, and a second domain.

Said first domain of said fusion molecule is HLA (Human Leukocyte associated Antigen) class II alpha chain, also referred to as HLA-DR alpha 2 domain, or a fragment thereof.

Specialized forms thereof are shown in the Figures and described in Examples 1 to 4. HLA class II is a heterodimer of two transmembrane glycoproteins, the alpha and beta chains. Oriented with their amino terminal ends on the outside of the cell, both chains comprise two extracellular domains, each of 90 - 100 amino acids, connected to a short cytoplasmic tail by a hydrophobic amino acid sequence that makes a single pass through the cell membrane. In the alpha chain the membrane distal domain is known as alpha 1 and the membrane proximal domain as alpha 2. Likewise, in the beta chain the membrane distal domain is known as beta 1 and the membrane proximal domain as beta 2. Both membrane proximal domains possess structural characteristics of C1-type immune globulin domains. The alpha 1 and beta 1 domains are polymorph and occupied with presentations of peptides (12 - 24mers) to the T-cell receptor during the course of T-cell activation. Studies using site-specific mutants have mapped the site of CD4 binding to the membrane proximal beta 2 domain of the HLA class II molecule.

HLA-DR alpha is conserved in humans and has not been recognized as a ligand for other molecules. The CD4 molecule interacts with the beta 2 domain of HLA class II (Marsh et al., *The HLA Facts Book* (2000)). The T-cell receptor binds to polymorphic beta 1 domains of HLA-DR with associated peptide antigen (Parham, *Immunological Reviews* 171 (1999), 1). Other molecules which bind to HLA-DR include super antigens such as staphylococcus enterotoxin B (SEB) (Li et al., *Ann. Rev. Immunol.* 17 (1999), 435; Jardetzky et al., *Nature* 368 (1994), 711) and toxic shock syndrome toxin (TSST-1) (Karp et al., *Nature* 346 (1990), 474; Kim et al., *Science* 266 (1994), 1870). These molecules bind the alpha 1 and beta 1 domains of HLA-DR molecules (Hargreaves et al., *Eur. J. Immunol.* 25, (1995), 3437).

Expression of certain HLA class II molecules and their polymorphism are strongly associated with a number of diseases such as insulin-dependent diabetes mellitus, Goodpasture syndrome, Pemphigus vulgaris, Systemic lupus erythramatosus, Multiple sclerosis, Grave's disease, Rheumatoid arthritis and Myasthenia gravis.

Of the particular importance is that a person's immune system develops tolerance to the self HLA class I and II allotypes expressed on the surface of that same person's cell. By contrast a person's immune system is not tolerant of the many hundreds of non-self HLA allotypes expressed by other human beings such as after organ transplantation. Therefore, once a person receives a transplant, hyperacute or acute rejection of the transplanted organ is likely to occur

if the recipient and donor are not compatible in their HLA antigen types expressed on the cell surface.

In one preferred embodiment of the present invention, said first domain and/or second domain, and preferably the entire fusion molecule is a protein. Most preferably, said fusion molecule is in a soluble form.

In another preferred embodiment the second domain of said fusion molecule comprises an immunoglobulin molecule or a fragment thereof. Preferably said immunoglobulin is a human immunoglobulin or a fragment thereof. Most preferably said immunoglobulin fragment of the immunoglobulin is the Fc portion of the immunoglobulin, wherein in a particularly preferred embodiment said Fc portion is the Fc portion of immunoglobulin G1 (IgG1). The use of the Fc portion of an immunoglobulin allows herein the elongation of the plasma half-life of the fusion molecule compared to the use of Fab fragments (Capon, et al., Nature 337 (1989), 525-531). Examples for immunomodulating fusion proteins are IL-10-Fc, wherein IL-10, an anti-inflammatory and antirejection agent has been fused to murine Fc[gamma]2a (Zheng et al., The Journal of Immunology 154 (1995), 5590-5600), tumor necrosis factor receptor linked with the Fc protein of human IgG 1 (Fisher et al., N. Engl. J. Med. 334 (1996), 1697-1702; Van Zee et al., The Journal of Immunology 156 (1996), 2221-2230), or fusion of Fc with CD4 receptor (Capon et al., Nature, 337 (1989), 525-531).

In connection with the present invention, it was surprisingly found that the HLA-DR alpha 2 domain, normally located on the cell surface of antigen-presenting cells (APC), could be generated in a recombinant form as shown in the appended Examples 1 and 2. Fused to entire immunoglobulins or fragments thereof, said recombinant HLA-DR alpha 2 fusion molecule is capable of modulating cytokine expression; i.e. it has been shown that soluble HLA-DR alpha 2-Fc fusion protein inhibited IFN- γ expression in PHA stimulated PBMCs, whereas there was no inhibition of IL-10 expression; see Example 3. Furthermore, the inhibitory effect of soluble HLA-DR alpha 2-Fc fusion protein on IFN- γ expression in stimulated immunocompetent cells was shown *in vivo* on splenocytes of LPS-induced mice; see Example 4. Keeping its immunomodulatory competence, the recombinant HLA-DR alpha 2 fusion

molecule induces modifications of cellular responses due to the corresponding receptor/ligand interaction.

Furthermore, in accordance with the present invention other immunoglobulin heavy chain constant regions can be used for said second domain of the fusion molecule of the present invention derived from any of the human IgG antibody subclasses referred to in the art as IgG1, IgG2, IgG3, and IgG4. Immunoglobulin heavy chain constant region domains have cross-homology among the immunoglobulin classes as described, for example, in US2004/082039. For example, nucleic acid sequences encoding, and amino acid sequences defining a human immunoglobulin Fc region, especially a Fc[gamma]1, Fc[gamma]2 and Fc[gamma]3, are used in the practice such as disclosed in WO00/40615, WO00/69913, WO00/24782 or in the Genbank and/or EMBL databases, for example, AF045536.1 (*Macaca fuscicularis*), AF045537.1 (*Macaca mulatta*), AB016710 (*Felix catus*), K00752 (*Oryctolagus cuniculus*), U03780 (*Sus scrofa*), 248947 (*Camelus dromedarius*), X62916 (*Bos taurus*), L07789 (*Mustela vison*), X69797 (*Ovis aries*), U17166 (*Cricetulus migratorius*), X07189 (*Rattus rattus*), AF57619.1 (*Trichosurus vulpecula*), or AF035195 (*Monodelphis domestica*). Furthermore, for experimental purposes, e.g., screening assays using mouse animal models, the fusion molecule of the present invention preferably comprises further the immunoglobulin molecules and fragments thereof, preferably the Fc portion of murine IgG subclasses known by the person skilled in the art as IgG1, IgG2a, IgG2b, IgG3.

In one preferred embodiment the first domain of said fusion molecule comprises the amino acid sequence depicted in SEQ ID NO: 1 (Fig. 1A and D) or a fragment thereof. Most preferably said fragment comprises amino acid sequence position 128 to 198 of SEQ ID NO: 1 as described in Example 2.

Said first and second domain of the fusion molecule of the present invention may be fused by covalent or non-covalent bonds. Therefore, the fusion molecule of the present invention may comprise, e.g., linker molecules. The linker could be made up of amino acids linked together by peptide bonds as described in WO/02066514. Alternatively, the present invention comprises chemical cross-linking of the above mentioned first and second domain, as described in Yang et al., Biochemistry 42 (2003), 3527-3535.

Principally, two domains could be fused without using a linker molecule as described above. Therefore, the Fc portion can be fused to a target protein or peptide via its C- or N-terminus using the N- and C-terminus of the protein, respectively. For example, a chimera of Fc and TNF and EPO is disclosed in EP 0 464 533, wherein the N-terminus of Fc was coupled to the C-terminus of the protein (X-Fc). The identical conjunction was selected for leptin-Fc chimeras as disclosed in WO97/00319 and WO97/24440.

The opposite linkage of Fc-protein chimeras (Fc-X), such as Fc-(IL-2), Fc-EPO, Fc-PSMA, Fc-(IL-12), Fc-TNF α , Fc-(GM-CSF), Fc-TNFR, Fc-endostatin, Fc, angiostatin, Fc-gp120, Fc-leptin, Fc-IFN α , Fc-(G-CSF) are described for example in WO96/08570, WO98/28427, WO99/02709 and WO99/58662. WO00/24782 discloses a huge number of possible Fc-X conjugates, wherein the linkage between the two partners may be Fc-X or X-Fc. An extensive development of Fc-X molecules was realized by Lexigen/Merck KgaA as disclosed in US-A-5,541,087, WO99/43713, WO99/29732, WO99/52562, WO99/53958, WO00/11033, WO01/07081, WO01/36489. Thus, X-Fc and Fc-X molecules which have "lost" their antigen binding sites, as well as molecules, wherein the binding sites and thus their antigen-specific targeting functions are conserved, are of great interest as promising therapeutic proteins, and there exists a further need to develop analogue compositions for different clinical applications. Non-natural therapeutic proteins are often particularly immunogenic. For example, Enbrel is a fusion protein consisting of an extracellular domain of a Tumor Necrosis Factor Receptor (TNF-R) fused to an Fc region of an antibody. All of those Fc fusion techniques can be applied in order to produce a fusion molecule of the present invention.

In another embodiment the present invention relates to a fusion molecule, which is encoded by a polynucleotide. As described above, said fusion molecule comprises in a first functional domain the HLA-DR alpha 2 chain, which is normally exposed on the cell surface of APC, forming a part of the MHC class II complex. The most important advantage of the present invention is to provide an experimental system which allows the recombinant expression and purification of said fusion molecule in a soluble and stable form as shown in Fig. 1 and 2 and described in the appended Examples 1 and 2. As the entire HLA-DR alpha 2 chain or a fragment thereof is combined with an entire immunoglobulin or a fragment thereof, in a recombinant fusion molecule, a experimental system for therapeutic and diagnostic needs could be provided. Moreover, the recombinant fusion molecule simplifies the elucidation of

the T-cell signaling pathway and therefore gives basis for lots of experimental purposes in questions concerning in the T-cell mediated immune response.

In another embodiment, the present invention relates to a polynucleotide encoding the fusion molecule of the present invention. The polynucleotide of the invention encoding the above-described fusion molecule 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, wherein said polynucleotide is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells. 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.

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. 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 a C- or N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant products. 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 (Invitrogen), or pSPORT1 (GIBCO BRL).

Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or 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 fusion protein may follow; see, Beychok, *Cells of Immunoglobulin Synthesis*, Academic Press, N.Y., (1979).

Furthermore, the present invention relates to vectors, particularly plasmids, cosmids, viruses and bacteriophages used conventionally in genetic engineering that comprise the polynucleotide encoding the fusion molecule of the invention. Preferably, the polynucleotide of said vector is operably linked to regulatory sequences allowing the transcription and optionally expression of said polynucleotide. Most 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 population. Methods which are well-known to those skilled in the art can be used to construct recombinant viral 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. (1994). 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*.

The present invention furthermore relates to a host cell transformed with a polynucleotide under the control of a heterologous promoter encoding said fusion protein or a vector of the invention. Said host cell may be a prokaryotic or eukaryotic cell. 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 host cell can be any prokaryotic or eukaryotic cell, such as a bacterial, insect, fungal, plant, animal or human cell. Preferred fungal cells are, for example, those of the genus *Saccharomyces*, in particular those of the species *S. cerevisiae*. The term "prokaryotic" is meant to include all bacteria which can be transformed or transfected with DNA or RNA molecules for the expression of a fusion protein 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, most preferably NSO and CHO cells. Depending upon the host employed in a recombinant production procedure, the fusion protein encoded by the polynucleotide of the present invention may be glycosylated or may be non-glycosylated. The fusion protein of the invention may also include an initial methionine amino acid residue. A polynucleotide 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. Furthermore, 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, NY, 1989). The genetic constructs and methods described therein can be utilized for expression of the antibody of the invention or the corresponding immunoglobulin chains 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. Suitable source cells for the DNA sequences and host cells for immunoglobulin expression and secretion can be obtained from a number of sources, such as the American Type Culture Collection ("*Catalogue of Cell Lines and Hybridomas*," Fifth edition (1985) Rockville, Maryland, U.S.A., which is incorporated herein by reference).

In another embodiment the present invention relates to a method for the production of a fusion molecule, or a biologically active fragment thereof, which comprises either culturing the host cell capable of expressing the fusion molecule of the present invention under conditions allowing for the expression of the fusion molecule, the *in vitro* translation of the polynucleotide encoding said fusion molecule or the crosslinking the domains and recovering the fusion molecule produced as described above.

Furthermore, the present invention relates to a fusion molecule which is obtainable by the above-mentioned method.

In a further aspect, the present invention relates to a pharmaceutical composition comprising the above-described fusion molecules, polynucleotides, vectors or host cells expressing said fusion molecule, and optionally to a pharmaceutically acceptable carrier.

The pharmaceutical composition of the present invention can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide) that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, tartaric, mandelic and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like. Particularly preferred is the HCl salt when used in the preparation of cyclic polypeptide [alpha]v antagonists. Physiologically tolerable carriers are well-known in the art. In a particularly preferred embodiment said pharmaceutical composition is in a soluble form. Exemplary of liquid carriers are sterile aqueous solutions that contain no materials in addition to the active ingredients and water, or contain a buffer such as sodium phosphate at physiological pH value, physiological saline or both, such as phosphate-buffered saline. Still further, aqueous carriers can contain more than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes. Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, and water-oil emulsions.

In one embodiment the present invention relates to a pharmaceutical composition for use in a broad variety of therapeutic needs, which concern preferably cell or organ transplantation, wound healing, the treatment of autoimmune, cardiovascular, allergic or infectious diseases, or the treatment of tumors. Examples of such disorders include, but are not limited to, an immune system disorder such as inflammation, actinic keratosis, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, arteriosclerosis, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, bursitis, cholecystitis, cirrhosis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, paroxysmal nocturnal hemoglobinuria, hepatitis, hypereosinophilia, irritable bowel syndrome, episodic lymphopenia with lymphocytotoxins, mixed connective tissue disease (MCTD), multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, myelofibrosis, osteoarthritis, osteoporosis, pancreatitis, polycythemia vera, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjgren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, primary thrombocythemia, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, trauma, and hematopoietic cancer including lymphoma, leukemia, and myeloma; a reproductive disorder such as a disorder of prolactin production, infertility, including tubal disease, ovulatory defects, and endometriosis, a disruption of the estrous cycle, a disruption of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, an endometrial or ovarian tumor, a uterine fibroid, autoimmune disorders, an ectopic pregnancy, and teratogenesis, cancer of the breast, fibrocystic breast disease, and galactorrhea, a disruption of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, impotence, carcinoma of the male breast, and gynecomastia; a nervous system disorder such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis,

brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease, prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome, fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system disorders, dermatomyositis and polymyositis, inherited, metabolic, endocrine, and toxic myopathies, myasthenia gravis, periodic paralysis, mental disorders including mood, anxiety, and schizophrenic disorders, akathisia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, and Tourette's disorder; a cell signaling disorder including endocrine disorders such as disorders of the hypothalamus and pituitary resulting from lesions such as primary brain tumors, adenomas, infarction associated with pregnancy, hypophysectomy, aneurysms, vascular malformations, thrombosis, infections, immunological disorders, and complications due to head trauma; disorders associated with hyperpituitarism including acromegaly, gigantism, and syndrome of inappropriate antidiuretic hormone (ADH) secretion (SIADH) often caused by benign adenoma; disorders associated with hypothyroidism including goiter, myxedema, acute thyroiditis associated with bacterial infection; disorders associated with hyperparathyroidism including Conn disease (chronic hypercalcemia); pancreatic disorders such as Type I or Type II diabetes mellitus and associated complications; disorders associated with the adrenals such as hyperplasia, carcinoma, or adenoma of the adrenal cortex, hypertension associated with alkalosis; disorders associated with gonadal steroid hormones such as: in women, abnormal prolactin production, infertility, endometriosis, perturbations of the menstrual cycle, polycystic ovarian disease, hyperprolactinemia, isolated gonadotropin deficiency, amenorrhea, galactorrhea, hermaphroditism, hirsutism and virilization, breast cancer, and, in postmenopausal women, osteoporosis; and, in men, Leydig cell deficiency, male climacteric phase, and germinal cell aplasia, hypergonadal disorders associated with Leydig cell tumors, androgen resistance associated with absence of androgen receptors, syndrome of 5 α -reductase, and gynecomastia; and a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective

tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus.

Preferably said pharmaceutical composition is designed to be administered to a subject. In a most preferred embodiment said subject is a mammal. In a further embodiment said pharmaceutical composition is adapted in a form to be administered orally, intravenously, subcutaneously, intramuscular or by inhalation. The appropriate concentration of the therapeutic agent might be dependent on the particular agent. The therapeutically effective dose has to be compared with the toxic concentrations; the clearance rate as well as the metabolic products play a role as do the solubility and the formulation. Therapeutic efficacy and toxicity of compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50.

In a further embodiment the present invention relates to a diagnostic composition comprising the above-described fusion molecule, a polynucleotide or a vector encoding, or a host cell expressing said fusion molecule, and optionally suitable means for detection. For diagnosis and quantification of (poly)peptides, polynucleotides, etc. in clinical and/or scientific specimens, a variety of immunological methods as well as molecular biological methods, like nucleic acid hybridization assays, PCR assays or DNA Enzyme Immunoassays (Mantero et al., Clinical Chemistry 37 (1991), 422-429) have been developed and are well known in the art. In this context, it should be noted that ligand fusion molecules such as HLA class II alpha 2 chain nucleic acid molecules may also comprise PNAs, modified DNA analogs containing amide backbone linkages. Such PNAs are useful, *inter alia*, as probes for DNA/RNA hybridization. The above-described diagnostic composition may be used for methods for detecting expression of HLA class II alpha 2 chain polynucleotide by detecting the presence

of mRNA coding for a HLA class II alpha 2 chain (poly)peptide which comprises, for example, obtaining mRNA from cells of a subject and contacting the mRNA so obtained with a probe/primer comprising a nucleic acid molecule capable of specifically hybridizing with a HLA class II alpha 2 chain polynucleotide under suitable hybridization conditions, and detecting the presence of mRNA hybridized to the probe/primer. Further diagnostic methods leading to the detection of nucleic acid molecules in a sample comprise, e.g., polymerase chain reaction (PCR), ligase chain reaction (LCR), Southern blotting in combination with nucleic acid hybridization, comparative genome hybridization (CGH) or representative difference analysis (RDA). These methods for assaying for the presence of nucleic acid molecules are known in the art and can be carried out without any undue experimentation.

Furthermore, the invention comprises methods of detecting the presence of a HLA class II alpha 2 chain protein in a sample, for example, a cell sample, which comprises obtaining a cell sample from a subject, contacting said sample with one of the aforementioned antibodies under conditions permitting binding of the antibody to the HLA class II alpha 2 chain protein, and detecting the presence of the antibody so bound, for example, using immuno assay techniques such as radioimmunoassay or enzymeimmunoassay. Furthermore, one skilled in the art may specifically detect and distinguish polypeptides which are functional HLA class II alpha 2 chain proteins from mutated forms which have lost or altered their HLA class II alpha 2 chain activity by using an antibody which either specifically recognizes a (poly)peptide which has HLA class II alpha 2 chain activity but does not recognize an inactive form thereof or which specifically recognizes an inactive form but not the corresponding polypeptide having HLA class II alpha 2 chain activity. The above described diagnostic compositions and methods can preferably be used to diagnose a disease or disorder as mentioned herein before.

These and other embodiments are disclosed and encompassed by the description and examples of the present invention. Further literature concerning any one of the materials, methods, uses and compounds to be employed in accordance with the present invention may be retrieved from public libraries and databases, using for example electronic devices. For example the public database "Medline" may be utilized, which is hosted by the National Center for Biotechnology Information and/or the National Library of Medicine at the National Institute of Health. Further databases and web addresses, such as those of the European

Bioinformatics Institute (EBI), which is part of the European Molecular Biology Laboratory (EMBL) are known to the person skilled in the art and can also be obtained using internet search engines. An overview of patent information in biotechnology and a survey of relevant sources of patent information useful for retrospective searching and for current awareness is given in Berks, TIBTECH 12 (1994), 352-364.

The above disclosure generally describes the present invention. Several documents are cited throughout the text of this specification. The contents of all cited references (including literature references, issued patents, published patent applications as cited throughout this application and manufacturer's specifications, instructions, etc.) are hereby expressly incorporated by reference; however, there is no admission that any document cited is indeed prior art as to the present invention.

A more complete understanding of the present invention can be obtained by reference to the following specific examples, which are provided herein for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLES

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art.

Methods in molecular genetics and genetic engineering are described generally in the current editions of Molecular Cloning: A Laboratory Manual, (Sambrook et al., (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press); DNA Cloning, Volumes I and II (Glover ed., 1985); Oligonucleotide Synthesis (Gait ed., 1984); Nucleic Acid Hybridization (Hames and Higgins eds. 1984); Transcription And Translation (Hames and Higgins eds. 1984); Culture Of Animal Cells (Freshney and Alan, Liss, Inc., 1987); Gene Transfer Vectors for Mammalian Cells (Miller and Calos, eds.); Current Protocols in Molecular Biology and Short Protocols in Molecular Biology, 3rd Edition (Ausubel et al., eds.); and Recombinant DNA Methodology (Wu, ed., Academic Press). Gene Transfer Vectors For Mammalian Cells (Miller and Calos, eds., 1987, Cold Spring Harbor

Laboratory); Methods In Enzymology, Vols. 154 and 155 (Wu et al., eds.); Immobilized Cells And Enzymes (IRL Press, 1986); Perbal, A Practical Guide To Molecular Cloning (1984); the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Immunochemical Methods In Cell And Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook Of Experimental Immunology, Volumes I-IV (Weir and Blackwell, eds., 1986). Reagents, cloning vectors, and kits for genetic manipulation referred to in this disclosure are available from commercial vendors such as BioRad, Stratagene, Invitrogen, and Clontech. General techniques in cell culture and media collection are outlined in Large Scale Mammalian Cell Culture (Hu et al., Curr. Opin. Biotechnol. 8 (1997), 148); Serum-free Media (Kitano, Biotechnology 17 (1991), 73); Large Scale Mammalian Cell Culture (Curr. Opin. Biotechnol. 2 (1991), 375); and Suspension Culture of Mammalian Cells (Birch et al., Bioprocess Technol. 19 (1990), 251); Extracting information from cDNA arrays, Herzog et al., CHAOS 11, (2001), 98-107.

Example 1: Cloning, expression and purification of a HLA-DR alpha 2 fusion molecule

The ability of HLA-DR alpha 2 to interact with its receptor(s) can be analyzed in binding studies utilizing a soluble HLA-DR alpha 2 fusion protein. For this purpose, the HLA-DR alpha 2 domain (Fig. 1A) was cloned into an insect expression vector containing the V5-protein cDNA and expressed as a fusion protein in insect cells to establish DES-expression system for HLA-DR alpha 2-V5 fusion protein expression, HLA-DR alpha 2-V5 fusion protein was generated using the DES-Drosophila expression system (Invitrogen). The HLA-DR alpha 2 extracellular domain (amino acids 132-188) was cloned into the pMTBiP/V5-His A, a vector containing the V5-epitope and a His-tag for purification. HLA-DR alpha 2-V5 fusion protein expression vector was stably transfected into Schneider 2 (S2) cells with calcium phosphate precipitation and selected by co-transfection with a blasticidin selection vector. Protein secretion was induced by addition of copper sulfate and medium supernatant was taken for protein purification. Fusion protein was purified using Ni²⁺-columns. For silver staining, HLA-DR alpha 2-V5 fusion proteins were purified from cell culture supernatant. 1 mg/lane was loaded on denaturing SDS polyacrylamid gels. The gels were stained with silver nitrate according to the standard protocol (Fig. 1B). For Western blotting HLA-DR alpha 2-V5 fusion proteins were separated with SDS-PAGE and blotted to nitrocellulose filter. The filter was blocked with 5% nonfat dry milk in 5mM Tris-buffered saline, pH 7.5,

with 0.05% Tween 20. Blots were probed with the indicated primary Abs at 1:200. The last step was followed by an appropriate secondary Ab conjugated to horseradish peroxidase at 1:1000 and proteins were visualized by chemiluminescence (ECL Detection Kit, Amersham Pharmacia) or when using an AP-conjugated secondary Ab proteins were detected using BCIP/NBT (Sigma) method (Fig. 1C).

Example 2: Cloning, expression and purification of a HLA-DR alpha 2-Fc fusion molecule

To perform further analysis the alpha 2 domain of HLA-DR was fused to human IgG1 Fc protein utilizing a mammalian expression vector system. For construction of HLA-DR alpha 2-Fc fusion protein a mammalian expression vector was developed in which the IGC1 domain of HLA-DR alpha 2 chain is expressed in its whole integrity (amino acids 128-198) fused to the human IgG1-Fc fragment. For the leader sequence the IG kappa leader was chosen, that is recommended for the secretion of recombinant antibodies. For expression of HLA-DR alpha 2-Fc fusion protein, COS7 cells were transiently transfected with the mammalian expression vector for HLA-DR alpha 2-Fc fusion protein using Fugene6 Transfection Reagent (Roche). One day after transfection media was exchanged to OptiMEM I (Gibco). After an additional 96 h HLA-DR alpha 2-Fc fusion protein was purified by Sepharose A column. Previous attempts to generate a fusion protein utilizing the HLA-DR-V5 expression system resulted in a very low yield of the expressed protein in the supernatants secreted by the transfected cells. To overcome the low expression a new fusion protein was designed, containing the HLA-DR alpha 2 domain in its entirety coupled with a human IgG1 Fc protein previously proven to be efficiently expressed in a mammalian expression system (Fig. 1D). This expression vector was transfected into COS7 cells and supernatants were subjected to Western blot using an anti-Fc-protein specific antibody. This construct revealed a band on Western blot which matches the theoretical 45 kDa weight of the HLA-DR - Fc fusion protein (Fig. 1E and 1F).

Example 3: Biological activity of HLA-DR-alpha 2 fusion molecule

To examine the biological activity of the HLA-DR alpha 2 fusion molecules the effect of soluble HLA-DR alpha 2-Fc fusion protein on cytokine expression was studied in PHA activated human T-cells. For ELISA cytokine analysis, PBMC of human healthy donors were isolated according to the Ficoll-Paque density centrifugation protocol. 5×10^4 PBMC/well

were incubated with 1 $\mu\text{g/ml}$ PHA (Sigma) at 5% CO_2 , 37°C for 48 h in presence of HLA-DR peptides, control peptide or HLA-DR-Fc and Fc control respectively, in a total volume of 100 μl /well. Samples were run in triplicates on 96 well-microtiter-plates. The quantitation of IFN- γ , was measured in supernatants of PHA stimulated cells. Cytokine level was determined using the Cytoscreen® ELISA Kit (Biosource). As shown in Fig. 1G, soluble HLA-DR alpha 2-Fc fusion protein inhibited IFN- γ expression, whereas there was no inhibition of IL-10 expression. Soluble HLA-DR alpha 2 provides a selective negative regulatory signal on Th1 cells after activation of T-cells.

Example 4: Reduction of IFN-gamma positive cells after LPS induced sepsis and HLA-DR alpha 2-Fc treatment

To investigate the biological activity of HLA-DR alpha 2 fusion molecules *in vivo*, the effect of soluble HLA-DR alpha 2-Fc fusion protein on cytokine expression was studied on splenocytes of LPS induced mice. Therefore, Balb/C mice (10-14 weeks old) were intraperitoneally induced with 50 μg LPS on day 0. Immediately afterwards, 4 mice were intraperitoneally treated with HLA-DR alpha 2-Fc (200 μg in PBS) or as control group with human Fc (50 μg in PBS). After 24 h the mice were anesthetized with CO_2 and blood was taken for serum extraction. Afterwards the mice were killed and the spleens were removed and diluted in 10 ml PBS. The splenocytes were isolated with a cell strainer. The cells without clumps were transferred in 15 ml tubes and centrifuged for 6 min at 1100 rpm. The erythrocytes were lysed with a red-blood lysis buffer from Sigma. Afterwards 5×10^6 cells were taken for intracellular FACS staining. The cells were washed with PBS/3 % FCS and then incubated with Fc-block (1 $\mu\text{g}/1 \times 10^6$ cells) for 20 min. After a further washing step the cells were fixed and permeabilized with CellFix/Perm-solution 2 (1:10 in PBS) for 10 minutes. The cells were washed twice with PBS/3 % FCS. The staining occurred with anti-IFN- γ -PE-Cy7 (2 $\mu\text{g}/1 \times 10^6$ cells) in PBS/ 3% FCS or control antibody for 30 min at 4°C. After staining the samples were washed twice with PBS/3 % FCS and then measured on FACS. As shown in Fig. 2, soluble HLA-DR alpha 2-Fc fusion protein significantly inhibited IFN- γ expression in splenocytes of the LPS-induced mice.

Claims

1. A fusion molecule comprising a first domain comprising the HLA-DR alpha 2 domain or a fragment thereof, and a second domain.
2. The fusion molecule of claim 1, wherein said fusion molecule is a fusion protein.
3. The fusion molecule of claim 1 or 2, which is in a soluble form.
4. The fusion molecule of any one of claims 1 to 3, wherein the second domain comprises an immunoglobulin molecule or a fragment thereof.
5. The fusion molecule of any one of claims 1 to 4, wherein said immunoglobulin is a human immunoglobulin or a fragment thereof.
6. The fusion molecule of any one of claims 1 to 5, wherein said immunoglobulin fragment of the immunoglobulin is the Fc portion of the immunoglobulin.
7. The fusion molecule of claim 6, wherein said Fc portion is the Fc portion of immunoglobulin G1.
8. The fusion molecule of any one of claims 1 to 7, wherein said first domain comprises the amino acid sequence depicted in SEQ ID NO: 1 or a fragment thereof.
9. The fusion molecule of claim 8, wherein said fragment comprises amino acid position 128 to 198 of SEQ ID NO: 1.
10. The fusion molecule of any one of claims 1 to 9, wherein said domains are fused by covalent or non-covalent bonds.

11. The fusion molecule of any one of claims 1 to 10, wherein said fusion molecule is encoded by a polynucleotide.
12. A polynucleotide as defined in claim 11.
13. A vector comprising the polynucleotide of claim 12.
14. The vector of claim 13, wherein said polynucleotide is operably linked to regulatory sequences allowing the transcription and optionally expression of said polynucleotide.
15. A host cell comprising a polynucleotide of any one of claims 1 to 10 under the control of a heterologous promoter or the vector of claim 13 or 14.
16. A method for the production of a fusion molecule, or a biologically active fragment thereof comprising:
 - (a) culturing the host cell of claim 15 under conditions allowing for the expression of the protein;
 - (b) *in vitro* translation of the polynucleotide as defined in claim 12; or
 - (c) crosslinking the domains as defined in any one of claims 1 to 10;and recovering the fusion molecule produced in (a), (b) or (c).
17. A fusion molecule, obtainable by the method of claim 16.
18. A pharmaceutical composition comprising a fusion molecule of any one of claims 1 to 11 or 17, a polynucleotide of claim 12, a vector of claim 13 or 14, or a host cell of claim 15, and optionally a pharmaceutically acceptable carrier.
19. The pharmaceutical composition of claim 18, wherein said pharmaceutical composition is in a soluble form.

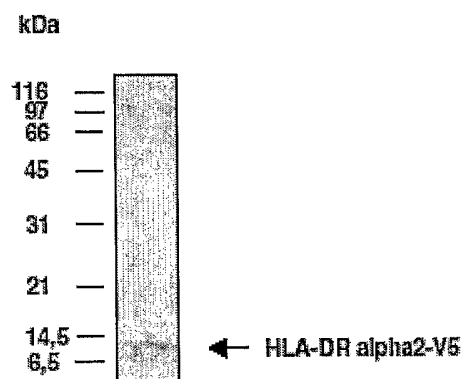
20. The pharmaceutical composition of claim 18 or 19 for use in cell or organ transplantation, for the treatment of autoimmune, cardiovascular, allergic or infectious diseases, or for the treatment of tumors.
21. Use of the pharmaceutical composition of claim 20 or 21, wherein said pharmaceutical composition is designed to be administered to a subject.
22. The use of claim 21, wherein said subject is a mammal.
23. The use of claim 21 or 22, wherein said pharmaceutical composition is adapted in a form to be administered orally, intravenously, subcutaneously, intramuscular or by inhalation.
24. A method of treating a subject suffering from a disease as defined in claim 20, comprising administering a fusion molecule of any one of claims 1 to 11, or 17, a polynucleotide of claim 12, a vector of claim 13 or 14, or a host cell of claim 15 in a therapeutically effective amount to the subject.
25. A diagnostic composition comprising a fusion molecule of any one of claims 1 to 11, or 17, a polynucleotide of claim 12, a vector of claim 13 or 14, or a host cell of claim 15, and optionally suitable means for detection.

1/4

1A

1 maisgvpvlg ffliavlmsa qeswaikeeh viiqaefyln pdqsgefmfd fdgdeifhvd
 61 makketvwrl eefgrfasfe aggalaniav dkanleimtk rsnytpitnv ppevtvltns
 121 pvelrepnvl icfidkftpp vvnvtwlrng kpvttgvsset vflpredhlf rkfhylpflp
 181 stedvyderv ehwgldepll khwefdapsp lpettenvvc algltvglvg iigtifiik
 241 gvrksnaaer rgpl

1B



1C

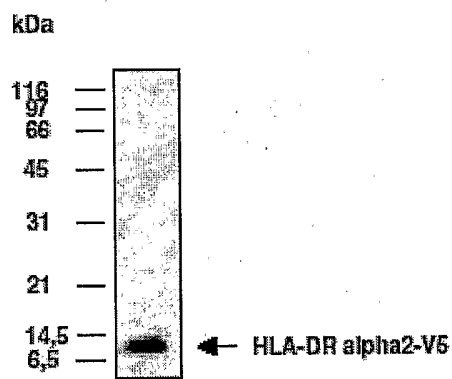


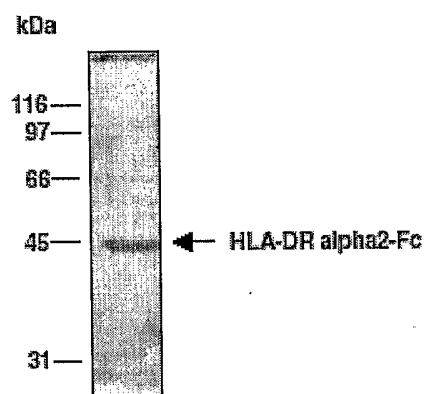
Fig. 1

2/4

1D

1 maisgvpvlg ffiiavlmsa qeswaikeeh viiqaeфыln pdqsgefmfd fdgdeifhvd
61 makketvwr1 eefgrfasfe aqgalaniav dkanleimtk rsnytpitnv ppevtvltns
121 pvelrepnl icfidkftpp vvnvtwlrng kpvttgvset vflpredhlf rkfhylpflp
181 stedvydarv ehwqldepll khwefdapsp lpettenwvc algltvqlvg iiigtifiik
241 gvrksnaaer rgpl

1E



1F

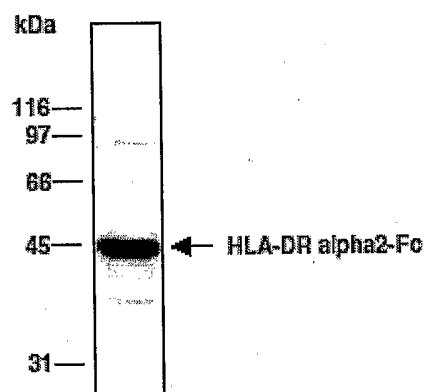
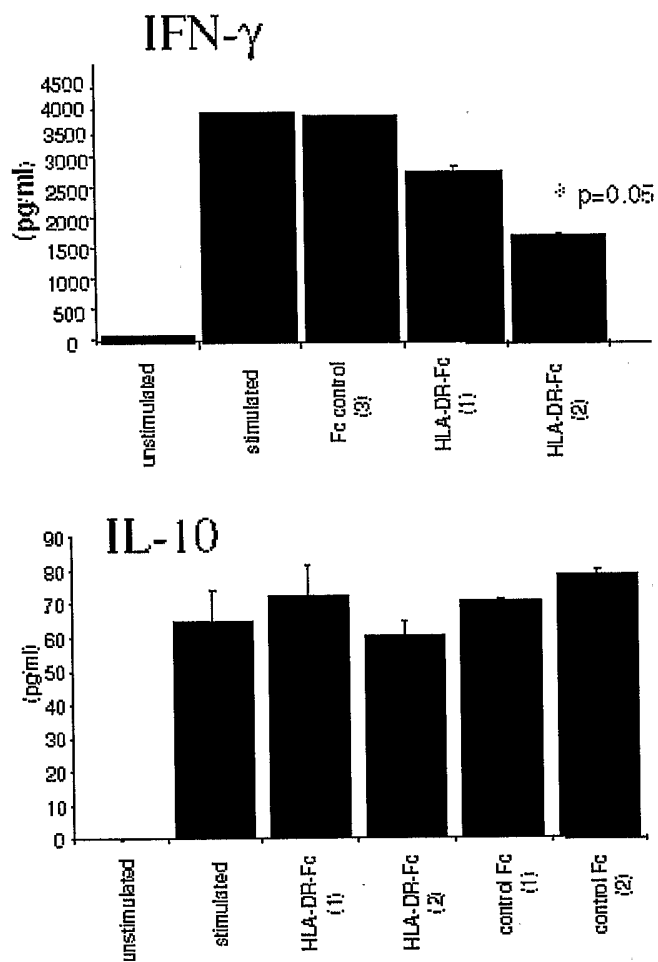


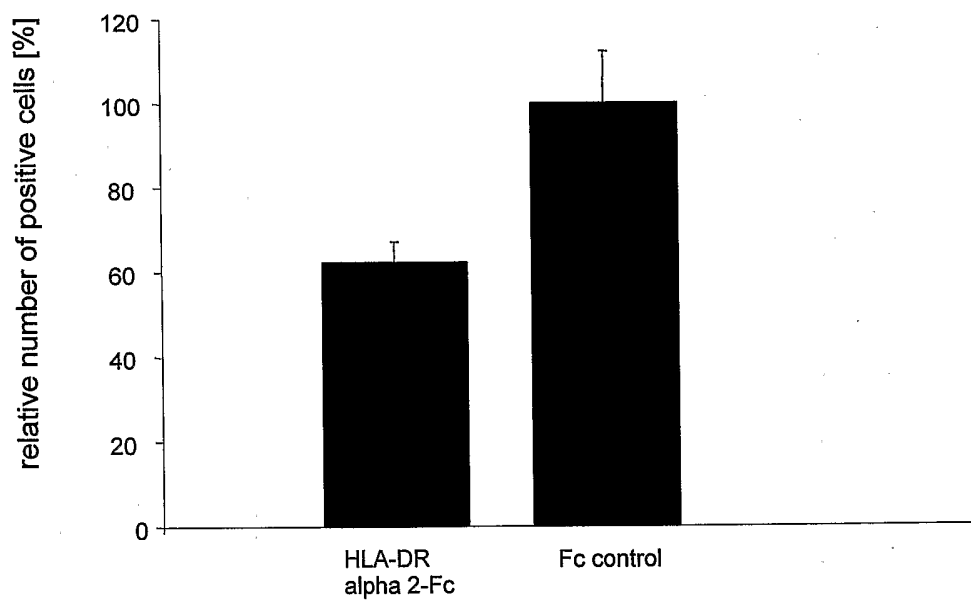
Fig. 1 (continued)

3/4

1G

**Fig. 1 (continued)**

4/4

**Fig. 2**

Sequence listing

<110> UTKU, Nalân

<120> HLA fusion molecules and uses thereof

<130> GE19A60/P-WO

<150> 60/645,992

<151> 2005-01-21

<160> 1

<170> PatentIn version 3.3

<210> 1

<211> 254

<212> PRT

<213> Homo sapiens

<400> 1

Met Ala Ile Ser Gly Val Pro Val Leu Gly Phe Phe Ile Ile Ala Val
 1 5 10 15

Leu Met Ser Ala Gln Glu Ser Trp Ala Ile Lys Glu Glu His Val Ile
 20 25 30

Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met
 35 40 45

Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys
 50 55 60

Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu
 65 70 75 80

Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu
 85 90 95

Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn Val Pro Pro
 100 105 110

Glu Val Thr Val Leu Thr Asn Ser Pro Val Glu Leu Arg Glu Pro Asn
 115 120 125

Val Leu Ile Cys Phe Ile Asp Lys Phe Thr Pro Pro Val Val Asn Val
 130 135 140

Thr Trp Leu Arg Asn Gly Lys Pro Val Thr Thr Gly Val Ser Glu Thr
 145 150 155 160

Val Phe Leu Pro Arg Glu Asp His Leu Phe Arg Lys Phe His Tyr Leu
165 170 175

Pro Phe Leu Pro Ser Thr Glu Asp Val Tyr Asp Cys Arg Val Glu His
180 185 190

Trp Gly Leu Asp Glu Pro Leu Leu Lys His Trp Glu Phe Asp Ala Pro
195 200 205

Ser Pro Leu Pro Glu Thr Thr Glu Asn Val Val Cys Ala Leu Gly Leu
210 215 220

Thr Val Gly Leu Val Gly Ile Ile Ile Gly Thr Ile Phe Ile Ile Lys
225 230 235 240

Gly Val Arg Lys Ser Asn Ala Ala Glu Arg Arg Gly Pro Leu
245 250