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(54) Title: REAGENTS AND METHODS FOR TREATING RHEUMATOID ARTHRITIS

(57) Abstract

The present invention is directed to agents for and methods of treating rheumatoid arthritis. Antibodies against MHC HLA DQw2 are administered to DQw2 patients with rheumatoid arthritis. Alternatively, peptides are developed that mimic the self peptides presented to the T-lymphocytes of these patients. These peptides prevent T cell activation stimulated by self peptides derived from DQw2 itself, Vα 12.1, an allele whose expression is found on expanded T cell clones of these patients, and other autologous antigens.

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REAGENTS AND METHODS FOR TREATING RHEUMATOID ARTHRITIS

Background of the Invention

Field of the Invention

This invention is directed to reagents which are capable of preventing, suppressing, or treating immune-related disease such as autoimmune disease. Specifically, the invention is directed to peptides derived from major histocompatibility complex HLA molecules, T cell receptors, and other autologous antigens responsible for autoimmunity in rheumatoid arthritis. The invention is also related to antibodies directed against the MHC antigen-presenting molecule.

Description of the Background Art

The Human Major Histocompatibility HLA Complex

The HLA system of antigens is controlled by genes located on the short arm of chromosome 6 in the major histocompatibility complex (MHC) region. This region has well-defined counterparts in other mammals such as mouse, dog, horse, pig, rat, and guinea pig. The HLA antigens are composed of two major classes: class I and class II. Class I and class II antigens differ in their biochemistry, cellular distribution, expression, and function. For a detailed examination of these features, see Dorf, M.E., ed., *The Role of the Major Histocompatibility Complex in Immunobiology*, New York: Garland STPM Press, 1981; Sullivan, K.A., *et al.*, The HLA System and Its Detection," in: Rose, N.R., *et al.*, eds., *Manual of Clinical Laboratory and Immunology*, pp. 835-846, Washington, DC, American Soc. for Microbiology, 1986.

The major distinctions between class I and class II molecules are as follows. Class I molecules consist of a 44,000 kd heavy chain noncovalently bound to β-2-microglobulin with a weight of 11,700 kd, whereas class II molecules consist of a 33,000 kd α chain that is relatively invariant and a 29,000 kd β chain that is responsible for most of the polymorphisms. Class

I molecules are ubiquitously distributed and appear on the surface of all cells, whereas class II molecules are restricted to B-lymphocytes, monocytes, macrophages, dendritic cells, Langerhans cells of the skin, and activated T cells. Class I molecules transport endogenous antigens, such as viral antigens, through the Golgi apparatus to the cell surface, whereas class II molecules transport endocytosed exogenous antigens, such as bacterial antigens, through the lysosomal/endosomal system. Class I molecules interact primarily with cytotoxic ($CD8^+$) T-cells, whereas class II molecules interact with helper ($CD4^+$) T-lymphocytes (Bjorkman, P.J., *et al.*, *Ann. Rev. Biochem. 59*:253-288 (1990)).

The genetic infrastructure of the HLA region as recently defined by the 10th International Histocompatibility Testing Workshop (Dupont, B., ed., *Histocompatibility Testing*, Springer-Verlag, New York (1987); Bodmer, W.E., *et al.*, *Bulletin WHO* (1987)) are shown in Figure 1. The class II genes encoding HLA DQ antigens are relevant to the present invention, since the association with autoimmune disease presented herein is with this HLA antigen. The HLA D region on the short arm of chromosome 6 has been subdivided into several subregions, including DR, DQ and DP. The subdivision is classically defined by serologic and cellular typing methods, and recently has been defined by biochemical and molecular analyses.

At each locus, one of several alternatives (alleles) of a gene may be found. Officially recognized alleles at each locus are designated by the locus and a number; thus, HLA-A1 is the 1 allele at the HLA-A locus. Alleles that have been tentatively assigned to a given locus but are not yet officially recognized are designated by a "w" (for "workshop") placed before the number, for example, HLA-DQw2. Official recognition results in the elimination of the "w" (e.g., HLA-DQ1).

The HLA system is extremely polymorphic, having multiple different alleles at each known locus. For example, there are at least 23 distinct alleles at the HLA-A locus, and at least 47 distinct alleles at the HLA-B locus. Each allele determines a product. The products of the HLA-A, -B, -C, -D, -DR,

-DQ and -DP alleles are cell surface molecules that bear the antigenic determinants.

Because of their close linkage, the combination of alleles at each locus on a single chromosome is usually inherited as a unit. This unit is referred to as the haplotype. Since one chromosome is inherited from each parent, there are two HLA haplotypes. Because all HLA genes are co-dominant, both alleles at a given HLA locus are expressed, and two complete sets of HLA antigens can be detected on cells. The HLA type of an individual is usually given as the phenotype and designates all HLA antigens possessed by the individual, for example, HLA-A1, -A2, -B7, -B12, -Cw1, -Cw2, -Dw2, -Dw3, -DR2, -DR3, -DQw1, -DQw2, -DRw52, -DPw1, and -DPw2.

The DQ α and β chains have 234 and 229 amino acids, respectively. Both the DQ α and β chains, in contrast to DR molecules, are highly variable. The DQ genomic subregion contains two α chain genes and two β chain genes.

T Cell Receptor

The thymus derived lymphocytes (T cells) mediate two general types of immunologic functions: effector and regulatory. Effector functions include delayed hypersensitivity, allograft rejection, tumor immunity, and graft-versus-host reactivity. These effector functions reflect the ability of T lymphocytes to secrete proteins called lymphokines, and the ability to kill other cells (referred to as "cytotoxicity"). The regulatory functions of T cells are represented by their ability to amplify cell-mediated cytotoxicity by other T cells and immunoglobulin production by B cells.

T cells are antigen-specific. The specificity is the result of receptor molecules that are capable of recognizing antigen. The receptor consists of two chains: an acidic α chain (molecular weight 45,000-55,000) and a more basic β chain (molecular weight 40,000-50,000), which are linked by a disulfide bond on the T cell surface. Both α and β chains are integral membrane proteins.

Both chains can be divided into variable and constant region domains. The variable region interacts with antigen, and thus the amino acid sequences of the variable region of different antigen-specific T cell clones are different. The amino acid sequences of the constant region from these same clones are similar. This general structure is analogous to the variable and constant region domains noted for immunoglobulin molecules.

The genetic loci encoding the T cell receptor genes can be divided into the following regions: variable region genes (V), diversity segment genes (D), joining region genes (J), and constant region genes (C). The V, D and J regions represent gene clusters. There are approximately 100 different V region genes for the α chain and around 80 different V region genes for the β chain. There are at least two D region genes for the β chain. There are around 100 J region genes for the α chain and 13 J region genes for the β chain. There is 1 C region gene for the α chain and 2 C region genes for the β chain. Rearrangements occur among these genes in a fashion analogous to that demonstrated for immunoglobulin genes. This provides the diversity to account for the existence in each individual of more than 1 million distinct T cell clones, each of which has a different antigen specificity.

The α/β T cell antigen receptor heterodimer does not recognize soluble antigen alone. The antigen receptor recognizes antigen in conjunction with products of MHC genes. In the case of soluble antigens, recognition occurs in conjunction with class II molecules. For viral antigens, recognition is in conjunction with class I molecules. Further, large soluble antigens are processed by an appropriate accessory cell, such as a macrophage or dendritic cell.

The sequence of events involved in T cell recognition of antigen is as follows. The antigen is phagocytosed by an antigen-presenting cell, internalized, processed, and then a peptide is expressed on the cell surface in conjunction with class I or class II MHC molecules. The T cell antigen receptor heterodimer then recognizes the antigen plus the MHC gene product. Recognition of antigen alone or MHC gene product alone is not sufficient to

signal T cell activation. Only the complex can be appropriately recognized by the T cell antigen receptor heterodimer.

The events required for T cell activation are as follows. Interaction between the antigen receptor complex on the T cell and antigen plus MHC gene products on accessory cells generates biologically active metabolites from membrane inositol. Another signal, initiated by interleukin-1, is sometimes required. In order for T cell activation (as measured by T cell proliferation) to proceed to completion, secretion of interleukin-2 must occur, and enhanced expression of the receptors for interleukin-2 must also occur. Interleukin-2 acts as an autocoid as it expands the particular T cell clone. Once T cells are activated by the signals required for antigen-specific activation, they can release lymphokines. These lymphokines act in an antigen-nonspecific fashion on other populations of mononuclear cells.

Autoimmunity

The central dogma of immunology has been that the immune system does not normally react to self. This phenomenon, described originally by Ehrlich, is accepted today as immunologic tolerance to self components, an obvious necessity for health. Accordingly, autoimmunity defines a state in which the natural unresponsiveness or tolerance to self terminates. As a result, antibodies or cells react with self constituents, thereby causing disease.

There is as yet no established unifying concept to explain the origin and pathogenesis of the various autoimmune disorders. Studies in experimental animals support the notion that autoimmune disease may result from a wide spectrum of genetic and immunologic abnormalities which differ from one individual to another and may express themselves early or later in life depending on the presence or absence, respectively, of many superimposed exogenous or endogenous accelerating factors. The disease process may be caused, among other things, by sensitized T lymphocytes. These lymphocytes produce tissue lesions by poorly understood mechanisms which may involve the release of destructive lymphokines or which attract other inflammatory

cells to the lesion. For a review of autoimmunity, see Theofilopoulos, A.N., in *Basic and Clinical Immunology*, chapter 11, Stites, D.P., *et al.*, 6th ed., Appleton & Lang, 1987.

Rheumatoid arthritis, a major autoimmune disease, is characterized by the presence in serum of autoantibodies directed against the Fc portion of IgG. Such autoantibodies, usually of IgM or IgG isotype, combine with IgG to form immune complexes that are considered to participate in the associated synovitis and vasculitis via activation of the complement cascade and attraction of polymorphonuclear cells to the sites of their deposition. Some rheumatoid factors cross-react with other autoantigens, such as DNA, histones, and cytoskeletal elements.

In rheumatoid arthritis, the local site of tissue injury is the joint. Joint pathology, characterized by inflammation and joint destruction, is the result of a complex interaction of cellular events (inflammatory cells, immunocompetent cells, and synovial lining cells) and their secreted products (Zvaifler, *Am. J. Med. 75*:3 (1983)). The synovial tissue (pannus) in rheumatoid arthritis has the appearance of a hypercellular lymphoid organ in which the predominant lymphocytes are T cells, which make up 80% of the synovial tissue lymphocytes (Bankhurst *et al.*, *Arthritis and Rheumatism 19*:555 (1976); Kurosaka, *J. Exp. Med. 158*:1191 (1983)).

In addition to the preponderance of T cells in the synovial pannus and an increased number of these T cells being activated *in vivo*, evidence for defects in T cell function and proportions have been described. Importantly, therapeutic measures that alter T cell function, such as total nodal lymphoid irradiation, significantly improve the clinical disease state, but are associated with marked toxicity (Kotzin *et al.*, *N. Engl. J. Med. 305*:969 (1981)).

Autoimmunity and MHC

HLA molecules and T cell receptors allow the immune system to distinguish self from foreign antigens through a selection process occurring in

the thymus (Dorf, M.E., ed., *The Role of the Major Histocompatibility Complex in Immunobiology*, New York: Garland STPM Press, 1981; Sullivan, K.A., *et al.*, The HLA System and Its Detection," in: Rose, N.R., *et al.*, eds., *Manual of Clinical Laboratory and Immunology*, pp. 835-846, Washington, DC, American Soc. for Microbiology, 1986; Braun, W.E., *Clin. Biochem. 25*:187-191 (1992)). It appears that differences in the amino acid sequences of HLA molecules, different capabilities of binding different peptide antigens in the interhelical groove, capability of presenting these antigens to and interacting with the repertoire of T cell receptors, as well as aberrant expression, may influence the occurrence of autoimmune diseases (Braun, W.E., *Clin. Biochem. 25*:187-191 (1992)).

HLA alleles have been significantly associated with a number of diseases (Braun, W.E., *HLA and Disease: A Comprehensive Review,* Boca Raton, FL, CRC Press, 1979; Tiwari, J.L., *et al.*, *HLA and Disease*, New York, Springer-Verlag, 1985). These include HLA-DR2 in narcolepsy, HLA-B27 in ankylosing spondylitis, and sero-negative arthropathies and HLA-DR3 with -DR4 in diabetes mellitus.

Three general ways have been described in which class II structural polymorphism may mediate disease susceptibility (Nepom, G.T., *et al.*, *Ann. Rev. Immunol. 9*:493-525 (1991)). "Polymorphic residues in the class II molecule might differentially bind a putative autoantigen peptide and present it to the responding T lymphocytes. Alternatively, the spectrum of expressed T cell receptors might be different in individuals with different class II genotypes. Autoreactive T cell receptors capable of recognizing an autoantigen class II complex would more likely be selected by thymic epithelium which expressed the nonsusceptible class II antigen. The third immunologic mechanism invoked to explain HLA disease associations is molecular mimicry (Tiwari, J.L., *et al.*, *HLA and Disease*, New York, Springer-Verlag, 1985). The presence of class I residues shared with a bacterial antigen or class II residues shared with a viral pathogen could result in tolerance to such microbial epitopes, leading to an altered immune response

(Nepom, G.T., *et al.*, *Ann. Rev. Immunol. 9*:493-525 (1991)). "Alternatively, such homologous regions could serve as an autoantigenic target with an immune response to the (bacterium or) virus leading to attack of self-cells expressing relevant class (I or) II products" (Nepom, G.T., *et al.*, *Ann. Rev. Immunol. 9*:493-525 (1991)).

MHC and Rheumatoid Arthritis

The relative risk of rheumatoid arthritis is high in individuals who inherit certain major histocompatibility complex genes. While the molecular basis for this genetic predisposition is unknown, because the major function of the MHC is to present process antigens to T lymphocytes, it has been hypothesized that an environmental antigen or infection initiates an MHC-restricted immune response mediated at least initially by T cells in rheumatoid arthritis. Rheumatoid arthritis has strong associations with class II antigens, Dw4 as defined by homozygous typing cells (HTC), and DR4 defined serologically. Early studies showed that Dw4 was present in 48% of 119 patients with rheumatoid arthritis compared to just 14% of 111 controls for a relative risk of 5.23. From numerous studies, it was known that not all individuals with DR4 suffered from rheumatoid arthritis and that about 20-35% with rheumatoid arthritis did not have DR4 (Nepom, G.T., *et al.*, *Ann. Rev. Immunol. 9*:493-525 (1991); Tiwari, J.L., *et al.*, *HLA and Disease*, New York, Springer-Verlag, 1985). After identification of five DR4 subtypes by HTC (Dw4, Dw10, Dw13, Dw14, and Dw15), it became clear that Dw4 and Dw14 were two major subtypes associated with rheumatoid arthritis. In DR4 positive caucasian adults with seropositive rheumatoid arthritis, about 50% had DR4 and about 35% the Dw14 subtype of DR4 (Biasch, J.M., *et al.*, *N. Engl. J. Med. 322*:1836-41 (1990)). These findings are summarized as follows. In rheumatoid arthritis, DR4 and/or DR1 occur in 93% of rheumatoid arthritis patients. Of the five subtypes of DR4, only two (Dw4 and 14) occur in rheumatoid arthritis. There is no contribution of DQ to rheumatoid arthritis

because, for example, both DQw7 and DQw8 occur as frequently in controls as in rheumatoid arthritis. Amino acids 70-74 of the DR β chains of DR1 (Dw1), DR4 (Dw4), DR14 (Dw14), and DR15 (Dw15) are essentially the same as the segment of the gp110 protein of the Epstein-Barr virus (except for a minor substitution of arginine for lysine at position 71) (Braun, W.E., *HLA and Disease: A Comprehensive Review,* Boca Raton, FL, CRC Press, 1979).

For these reasons, scientific investigators, searching for the causative agent for rheumatoid arthritis, have postulated that immune disregulation associated with the disease may be associated with specific genes of the major histocompatibility complex. For these reasons also, it has been postulated that the disease may be associated with clonally expanded T cells detectable as a skewing of the peripheral α β T cell antigen receptor repertoire. U.S. Patent No. 4,886,783, for example, describes a method of diagnosing autoimmune diseases, including rheumatoid arthritis, based upon an expansion of Vβ gene usage in the total population of T lymphocytes in rheumatoid arthritis patients.

PCT International Publication No. 90/06758, which broadly covers monoclonal antibodies reactive with epitopes on the T cell antigen receptor that can be used in the diagnosis and treatment of many immune related diseases, associates rheumatoid arthritis with an increased percentage of T cells which express Vλ1, Vβ3, Vβ9, or Vβ10 T cell receptor variable regions in a patient sample. Methods of diagnosing and treating rheumatoid arthritis patients with monoclonal antibodies specific for these gene products are described.

Copending patent application 07/750,986 describes the detection of a subset of individuals afflicted with rheumatoid arthritis using a reagent having specificity for a unique sequence within the variable region of the α chain of the T cell receptor for antigen, and specifically for Vα12.1. The application discloses the expression of the Vα12.1 gene product on $CD8^+$ T lymphocytes that is expanded in a subset of rheumatoid arthritis patients when compared to the expression of Vα12.1 on $CD8^+$ lymphocytes of normal, healthy subjects that do not have the immune abnormality. This expression thereby enables the

detection of this subset of patients with reagents that bind specifically to Vα12.1 sequences. Accordingly, that application is directed to monoclonal antibodies reactive with an epitope of the α chain variable region Vα12.1 on human T lymphocytes.

Because it is now known that these and other autoimmune diseases involve the action of T cells stimulated by the binding of their T cell receptors to an MHC/autoantigen (or non-autoantigen) complex, prevention and/or treatment strategies have been proposed which are based on the disruption of interactions between the MHC/antigen complex and the TCR (Wraith, D.C., *et al.*, *Cell 57*:709-715 (1989)). Approaches based on this principle include vaccination with whole T cells, passive blockade using antibodies which bind to the TCR, passive blockade using antibodies that bind to the MHC portion of the complex, administration of antibodies reactive with the T cell markers (e.g., $CD4^+$, $CD8^+$), and the use of peptides which mimic the antigen of interest and compete for binding to the MHC or the TCR molecule.

T cells expressing the TCR αβ heterodimer can induce idiotypic and V gene family-specific antibodies that can regulate T cell function (Owhashi, M., *et al.*, *supra*; Gascoigne, N.R.J., *et al.*, *Proc. Natl. Acad. Sci. USA 84*:2936 (1987); Kappler, J.W., *et al.*, *Nature 332*:35 (1988); Kappler, J.W., *et al.*, *Cell 49*:263 (1987); MacDonald, H.R., *et al.*, *Nature 332*:40 (1988)). For example, antibodies that recognize the TCR Vβ8 sequence have been effective in the prevention and treatment of autoimmunity in mice and rats (Owhashi, M., *et al.*, *supra*; Acha-Orbea, H., *et al.*, *Cell 54*:263-273 (1988); Urban, J., *et al.*, *Cell 54*:577-592 (1988)). Obtaining such antibodies selective for V region gene products has been dependent upon the availability of T cell clones that express TCR encoded by the relevant V gene family, and requires a laborious screening procedure using whole cells to establish specificity.

While antibody therapies in which antibodies are directed to MHC molecules and CD4 molecules have been generally successful in several animal models of autoimmunity, these approaches may be too nonspecific and potentially overly suppressive, since 70% of T cells bear the $CD4^+$ marker,

and since all T cell-mediated responses and most antibody responses require MHC-associated antigen presentation.

I.R. Cohen's laboratory has developed an approach to the immunospecific treatment of autoimmunity which utilizes whole live or attenuated T lymphocytes as vaccines to treat or prevent EAE, experimental autoimmune thyroiditis (EAT), and experimental arthritis. This approach is reviewed in Cohen, I.R., *Immunol. Rev.* *94*:5-21 (1986), which discusses several animal models of autoimmune disease wherein vaccination with disease-specific T lymphocytes has been used to generate prophylactic or therapeutic effects. The fine specificity of vaccination was dictated by the fine specificity of the T cell recognition, possibly implicating the TCR. For example, two different anti-MBP T cell lines, each reactive to a different epitope of MBP, were found to vaccinate against EAE specifically induced by the particular epitope, indicating some form of anti-idiotypic immunity. However, when attempts were made to isolate clones of MBP-specific or thyroglobulin-specific T cells (in a thyroiditis model) from the non-clonal cell lines, only clones producing disease, but not resistance, were obtained. This led to the finding that appropriate aggregation or rigidification of cell membranes, by either hydrostatic pressure or chemical cross-linking, yielded cells which could induce protection more consistently. Similarly, low doses (sub-encephalitogenic) of MBP-specific cells could also induce resistance to lethal EAE. The protective state was termed "counter-autoimmunity." This state involves T cell clones which can specifically proliferate in response to the vaccinating T cells, can suppress effector clones *in vitro* (non-specifically, presumably through release of a suppressive lymphokine), and can adoptively transfer counter-autoimmunity *in vivo*. Such counter-autoimmunity is accompanied by suppressed delayed hypersensitivity (DH) responses to the specific epitope and prevention or remission of clinical disease.

A major difficulty with the foregoing approaches is that they require the use of complex biological preparations which do not comprise well-defined therapeutic agents. Such preparations suffer from complex production and

maintenance requirements (e.g., the need for sterility and large quantities of medium for producing large number of "vaccine" T cells), and lack of reproducibility from batch to batch. The T cell "vaccine" preparations, to be useful in humans, must be both autologous and individually specific, that is, uniquely tailored for each patient. Furthermore, the presence of additional antigens on the surface of such T cells may result in a broader, possibly detrimental, immune response not limited to the desired T cell clones (Offner, H., *et al.*, *J. Neuroimmunol. 21*:13-22 (1989)).

There is a great need, therefore, for agents and pharmaceutical compositions which have the properties of specificity for the targeted autoimmune response, predictability in their selection, convenience and reproducibility of preparation, and sufficient definition to permit precise control of dosage.

Summary of the Invention

The present invention is based upon the applicants' discovery that a subset of rheumatoid arthritis patients have a marked expansion of Vα12.1 bearing CD8+ T cells, and that a high frequency of these patients share the extended HLA DQw2 haplotype. Molecular analysis of the T cell receptors in each of three unrelated patients with Vα12.1 elevation revealed antigen-driven clonal expansions. Among the Vα12.1-elevated group of rheumatoid arthritis patients, the frequency of HLA DQw2 was 86%. Accordingly, the invention is directed to reagents that block the normal interaction of HLA DQw2 antigen with T cell receptors, and particularly the T cell receptors bearing the Vα12.1 rearrangement, and to methods of using the reagents.

The methods of the present invention are directed to administering DQw2 antibody to DQw2 rheumatoid arthritis patients and particularly rheumatoid arthritis patients bearing T cell receptors containing the Vα12.1 rearrangement. In further preferred embodiments of the invention, the

methods of the present invention use a monoclonal antibody against the DQw2 antigen.

Other reagents of the invention include peptides, preferably of around 8-24 amino acids, that represent alterations of the normally processed endogenous antigens that are causally related to autoimmunity in rheumatoid arthritis. These peptides may be derived from the DQw2 molecule itself which is being recognized as a "nonself" antigen and presented either by DQw2 itself as the MHC molecule on an effector cell or by another MHC antigen presenting molecule. Alternatively, the peptide may be derived from the T cell receptor Vα12.1 gene product.

When the autoantigen is established to be a portion of DQw2 itself, this portion is altered such that it is recognized by the DQw2 on the antigen presenting cell and either displaces the toxic autoantigen, prevents further binding of the DQw2 present on the antigen presenting cell with the toxic autoantigen, or otherwise interferes with T cell activation. This competition or displacement thus prevents the further activation of T cells which were specific for the offending autoantigen. Alternatively, the peptide may be derived from Vα12.1 and used for essentially the same purposes.

In alternative embodiments of the invention, the offending autoantigen may be recovered and the peptide sequenced. Subsequently, altered sequence variants of the relevant autoantigen peptide may be prepared to either displace or compete with the true endogenous offending autoantigen.

The peptides so prepared may then be used as a treatment to ameliorate the symptoms of rheumatoid arthritis in DQw2 patients with rheumatoid diseases, particularly rheumatoid arthritis.

The invention is further directed to nucleic acid sequences corresponding to the peptides of the present invention which can be cloned into appropriate expression vectors such that the peptides are produced in sufficient amounts to be therapeutically useful. Alternatively, the peptides can be synthesized by standard chemical synthetic procedures.

Brief Description of the Figures

Figure 1. Major histocompatibility complex HLA genes.

Figure 2. Expansion of Vα12 bearing T cells in rheumatoid arthritis patients. (A) Flow cytometric analysis was performed using directly conjugated antibodies. Heparinized blood was obtained from individuals and PBMC was isolated using Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) and suspended in staining buffer (PBS/5% human serum with 0.02% NaN_3) containing saturating amounts of conjugated mAbs and analyzed by a Facscan flow cytometer (Becton Dickinson) Two-color immunofluorescence analysis of Vα12 expression on $CD4^+$ and $CD8^+$ T cells was carried out on peripheral blood mononuclear cells (PBMC) using OKT4 (anti-CD4) or OKTB (anti-CD8) FITC conjugated mAb and anti-Vα12 mAb (6D6-PE) using a FACSCAN (Becton Dickinson and Co., Mountainview, CA). Lymphocytes were gated on the basis of forward and side scatter profiles (not shown) and analyzed for fluorescence intensity in log scale. Ten thousand viable cells were analyzed by gating on lymphocytes excluding propidium iodide. The data was analyzed by dividing the dot plot into quadrants to represent unstained cells (lower left, quadrant 3), cells stained with FITC alone (OKT4 or OKT8) (lower right, quadrant 4), cells stained with FE alone (6D6) (upper left, quadrant 1), and cells that were co-stained with FITC and PE (upper right, quadrant 2). (B) The Vα12 expression on $CD4^+$ and $CD8^+$ T cells from adult rheumatoid arthritis patients was determined using data generated for individual peripheral blood samples stained as in A. The expression of Vα12 on $CD4^+$ and $CD8^+$ subsets for each individual are connected by a line. The following formula was used to calculate the percentage value for Vαl2 expression: % $V\alpha l2^+/CD4^+$ (or $CD8^+$) cells = {$V\alpha12^+$ cells co-stained with anti-CD4 (or anti-CD8) (2nd quadrant)/% T cells that were $CD4^+$ (or $CD8^+$)} x 100.

Figure 3. Predicted amino acid sequences of the Vαl2 containing transcripts. Nucleotide sequences of Vα12.1 containing transcripts was

generated by direct (Vα12.1-specific) or by iPCR (all Vαs) and cloned into M13 vector. Separation of $CD4^+$ and $CD8^+$ T cells was carried out on Ficoll-Paque (Pharmacia Fine Chemicals," in Uppsala, Sweden) isolated PBMC and suspended in RPMI-1640 containing 5% FCS at $5x10^5$/ml to mix with saturating amounts of anti-CD4 (OKT4) or anti-CD8 (OKT8 and 89.2) mAb. First selection was carried out by rotating for 1 h at 4° C and washed away free antibody with RPMI/5% FCS before adding 1 to 2 fold excess goat anti-Mouse Ig conjugated Dynabeads (Dynabeads M-450, Dynal, Oslo, Norway), and cells bound to beads were removed and washed extensively before solubilizing in RNA lyses buffer. Under these conditions, $CD4^+$ or $CD8^+$ T cells are enriched to greater than 98% purity as verified routinely by staining the negatively selected populations by FACS (i.e., OKT4 or OKT8 depleted cell populations). Second step of selection with anti-Vα12.1 (mAb 6D6) was carried out on CD4-depleted (CD8-enriched) cells in order to isolate Vα12.1/$CD8^+$ T cells to make RNA and use in the iPCR method. Purification of RNA from $CD8^+$ or Vα12.1$^+$/$CD8^+$ T cells was carried out according to Chomczynski *et al.* (*Anal. Bioch. 162*:156 (1987). Total RNA was quantitated and analyzed for intactness by resolving on a 1% agarose mini-gel and visualized by staining with EtBr. Complementary DNA (cDNA) for the direct PCR method was synthesized in a reverse transcription reaction using reverse transcriptase and 1-5 ug of total RNA by priming with 500 ng of oligo-$(dT)_{12-18}$ primer. All of the Vβs, Vαs and Jαs gene segments present in the Vα12.1$^+$/$CD8^+$ T cells were identified by a second method that does not require prior sequence information on both ends of the DNA to be amplified. This technique independently confirmed findings generated by the direct PCR method, and provided new sequence information on Vα, Jα and chain usage. Inverse PCR (iPCR) was first established to amplify the flanking region of a known sequence within a given restriction fragment of genomic DNA. Recently, this method was shown to be applicable to the amplification of the full length cDNA of TCR α and β genes.

First strand cDNA was synthesized from RNA of T lymphocytes by priming with an olig-$(dT)_{12\text{-}18mer}$, using Moloney murine leukemia virus derived reverse transcriptase lacking RNase H activity. For second strand cDNA synthesis, RNase H was used to nick the mRNA strand of the RNA/DNA hybrid, generating a series of RNA primers that serve to synthesize the second strand DNA with *E. coli* DNA polymerase I and *E. coli* DNA ligase. The double-stranded cDNA was blunt ended using the 3'-5' exonuclease activity of T4 DNA polymerase and the DNA was then circularized using T4 DNA ligase. This circular cDNA was used as the template for the iPCR amplification by using a pair of Cα or Cβ primers, which are oriented in an outward direction from one another. This method generated PCR products of approximately 700 bp for the TCRα chain, and for the TCRβ chain, a major (700 bp) and a minor (approximately 400 bp) corresponding to the expected VDJCβ and DJCβ transcript lengths. This method generated up to $2x10^6$ primer specific cDNA clones starting with as little as 1 ug of total RNA (Uematsu, *Immunogen. 34*:174 (1991). A library could be generated of TCRα or cDNA products from RNA isolated from as little as $2x10^4$ of fresh peripheral T cells. One of the two primers contained an artificial Sal I site and the other primer contains a Not I site, which facilitated DNA cloning and sequencing. Restriction sites in the PCR primers were used to generate sticky ends for cloning. Appropriately sized DNA products were isolated from low melting point (LMP) agarose gels (BRL, Gaithersburg, MD) and ligated to vector with cloning sites. Cloning and sequencing were carried out by the dideoxy chain termination method using the modified T7 polymerase (Sequenase; United States Biochemical Corp., Cleveland, OH). The sequencing products were resolved on polyacrylamide gels and autoradiography was carried out according to standard methods.

Primers used in iPCR:

Cα-forward (SEQ ID NO: 1):

5'-GGGTCGACGACCTCATGTCTAGCACAGT (Sal I)

Cα-inverse (SEQ ID NO: 2):

5'-GCATGCGGCCGCCCTGCTATGCTGTGTGTCT (Not I)

Cβ-forward (SEQ ID NO: 3):

5'-GGGTCGACACACAGCGACCTCGGCTGGG (Sal I)

Cβ-inverse (SEQ ID NO: 4):

5'-GCATGCGGCCGCCATGGTCAAGAGAAAGGA (Not I)

All of the α-chains cloned and sequenced from the Vα12.1$^+$/CD8$^+$ T cells by iPCR did actually contain Vα12.1 gene segment.

Figure 4. Expanded Vα12$^+$ T cells in rheumatoid arthritis were previously activated. The cell surface expression of DR, IL-2R α and β chains, CD45RO, HLA-1 and transferrin receptor were analyzed by immunofluorescence staining as described in Figure 2. The graph represents relative expression amongst CD3$^+$, CD4$^+$, CD8$^+$ and Vα12$^+$ T cells. mAbs LB3 .1 (anti-HLA DR), 84.19.9 (anti-IL-2Rα), Tu27 (anti-IL-2R β), UCHL1 (anti-CD45RO), TS2/7 (anti-VLA-1), 5E9 (anti-transferrin receptor) were used at 1/100 ascites dilution with a second antibody (goat anti-Mouse immunoglobulin) conjugated with FITC or PE. The following formula was also used to calculate the percentage value for each activation antigen relative to Vα12.1$^+$ T cells: LB3.1 (or other activation antigens)/Vα12$^+$ (or CD4$^+$, or CD8$^+$) cells = (LB3.1$^+$ cells co-stained with anti-Vα12 (or anti-CD4 or anti-CD8) (2nd quadrant)/% T cells that were Vα12$^+$ (or CD4$^+$ or CD8$^+$)) x 100.

Figure 5. Serologic HLA typing on "α12" group of patients that were carried out the BWH HLA lab. No common class I was found to associate specifically with this group. HLA DQw2 allele, on the other hand, was identified in 5 of 6 patients from this group, while only on patient from this Vα12 expanded group lacked the HLA DQw2 allele. (B) Total of 26 patients were HLA typed serologically and separated into DQw2 positive or DQw2 negative panel. Five of 13 patients with HLA DQw2 allele had the striking Vα12 expansion within the CD8 subset of T cells, while only one of 13 patients that lacked HLA DQw2 allele had expanded Vα12 T cells.

Detailed Description of the Preferred Embodiments

It has now been found that there is a correlation between the presence of the Vα12.1 T cell receptor variable region and the propensity for autoimmune disease, rheumatoid arthritis, in a subset of patients with said disease. Furthermore, in these hosts which express this T cell variable region receptor rearrangement, antigen presenting cells carry the MHC DQw2 haplotype as the antigen presenting molecule. In view of this unexpected correlation, the present invention is directed to reagents and methods based on the human T cell receptor Vα12.1 rearrangement and the DQw2 haplotype for treating humans suffering from rheumatoid arthritis. Accordingly, an object of the present invention is to provide compositions, pharmaceutical formulations, and methods useful for treating humans suffering from rheumatoid arthritis. A still further object of the invention is to provide compositions, pharmaceutical formulations, and methods useful for preventing or attenuating the clinical symptoms of rheumatoid arthritis.

Accordingly, the invention is directed to a peptide comprising an amino acid sequence (I) that is analogous to an amino acid sequence (II) that is presented to a T lymphocyte, in rheumatoid arthritis patients, by an MHC-bearing antigen-presenting cell, wherein amino acid sequence II is derived from the DQw2 protein.

By "amino acid sequence (I)," for the purpose of the invention, is intended a sequence which is recognized by and bound to a major histocompatibility antigen on an antigen presenting cell. In rheumatoid arthritis patients, for the purpose of the invention, amino acid sequence (II) is a processed peptide derived from a naturally occurring protein in these patients which is treated by the patient's immune system as a nonself antigen, processed, and presented to a T lymphocyte by an MHC-bearing antigen presenting cell as a foreign antigen. This presentation thus activates specific T lymphocytes to complete an immune response against the patient's own protein. Accordingly, for the purpose of the present invention, amino acid

sequence I is designed to bind the major histocompatibility antigen present on the antigen presenting cell, displacing the processed self peptide or preventing the binding of the processed self peptide to the major histocompatibility molecule.

In alternative embodiments, the peptide of the present invention is derived from a persistent foreign antigen, including, but not limited to, viral antigens that are the result of a persistent viral infection. Accordingly, for the purpose of the present invention, amino acid sequence I is also designed to bind the major histocompatibility antigen present on the antigen presenting cell, and displacing the foreign antigen or preventing the binding of the foreign antigen to the major histocompatibility molecule or the functional interaction with the T-cell.

The peptide is also designed to bind to the MHC molecule but to prevent interaction of the MHC/antigen complex with a relevant T lymphocyte so as to prevent the activation and clonal expansion of this T lymphocyte. The alteration in the amino acid sequence of the MHC binding antigen is designed either to prevent the binding of the complex to the T cell receptor or to allow the binding of the receptor but to prevent further activation.

It is understood that the amino acid sequence comprising the peptide of this invention can be used alone, or bound to, or contained within the sequence of, a longer peptide. The longer peptide may carry additional sequences derived from the parent protein of interest or may include sequences of an unrelated peptide, such as a carrier protein, used to enhance the binding of the amino acid sequence of interest to the MHC antigen or to enhance the interaction of the complex with a T lymphocyte.

Accordingly, in one embodiment of the invention the peptide contains an amino acid sequence (I) derived from the MHC HLA DQw2 protein. Because this protein is expressed in the subset of rheumatoid arthritis patients with a clonal expansion of T cells bearing the Vα12.1 rearrangement, the invention is specifically directed to the use of the DQw2 derived amino acid sequence I in patients bearing the Vα12.1 clonal expansion. However, the use

of the peptide is not limited to such patients, but is presumed to be effective in treating any rheumatoid arthritis patient bearing the DQw2 haplotype.

In certain embodiments of the invention, the MHC protein is MHC HLA DQw2 itself. In these embodiments, the DQw2 peptide is presented to a T lymphocyte by the MHC HLA DQw2 present on an antigen presenting cell. According to the invention, amino acid sequence II, derived from DQw2, is thus presented by the DQw2 MHC protein. Accordingly, analogs of this peptide are designed to bind to DQw2 and to thus prevent interaction with or block activation of the T cell presented with the DQw2 peptide by DQw2 itself. In alternative embodiments of the invention, the MHC protein is other than DQw2 and may be any of the other relevant major histocompatibility proteins found on antigen presenting cells and which function to present processed DQw2 antigens to T lymphocytes.

In specific embodiments of the invention, the peptide is bound to HLA DQw2, which peptide stimulates Vα12.1-bearing T cells. This peptide may be derived from any self or foreign protein if it has the properties of stimulating Vα12.1-bearing T cells.

In further embodiments of the invention, the peptide contains an amino acid sequence I that is analogous to an amino acid sequence in the α chain of a T cell receptor which bears the Vα12.1 rearrangement.

In still further embodiments of the invention, the disease-causing peptide does not require alteration. Instead, the peptide portion which is presented by a major histocompatibility complex antigen on an antigen presenting cell, is derived from an appropriate protein but is not altered in amino acid sequence. Instead, the peptide is administered in the appropriate dose so as to induce tolerance, which entails diminished T cell activity.

Accordingly, the present invention is broadly directed to a method for preventing, ameliorating, or otherwise treating rheumatoid arthritis by the administration of any of the peptides described above in amounts sufficient for such amelioration, prevention, or treatment. The reagents of the present invention, when utilized in the methods of the present invention, may act on

a number of levels. Accordingly, the efficacy of the reagents includes, but is not limited to, preventing antigen presentation, preventing T cell activation, or inducing a state of anergy in the T cell population.

The present invention is thus directed to a method for preventing antigen presentation in patients with rheumatoid arthritis, said patients expressing the MHC HLA DQw2 allele, comprising administering any of the peptides described above in amounts sufficient to prevent the presentation. The invention is further directed to a method for preventing T cell activation in patients with rheumatoid arthritis, which patients express the MHC HLA DQw2 allele, comprising administering any of the peptides described above in amounts sufficient to prevent the activation. The methods of the present invention are also broadly useful for preventing or ameliorating a chronic inflammatory response in patients with rheumatoid arthritis and expressing the MHC HLA DQw2 allele, the response resulting from the persistent activation of T lymphocytes by an MHC-bearing antigen presenting cell, particularly where the MHC is the DQw2 gene product. The method comprises administering any of the proteins described above in amounts sufficient to prevent the chronic inflammatory response. The reagents and methods of the present invention are also useful to induce tolerance which entails diminished T cell activity. This tolerance is induced by the administration of peptides derived from the appropriate antigens but not bearing alterations in amino acid sequence that is different from the offending protein. In doing so, anergy, which is the inability of the recipient T cell to react to the antigen, is induced. The anergy results from the tolerization of the recipient to a particular antigen, which is induced by administration of excessive amounts of the antigen.

The peptide may also contain an amino acid sequence I derived from the α chain of a T cell receptor bearing the Vα12.1 rearrangement. In this embodiment, a peptide is prepared which is analogous to the portion of the Vα12.1 which is presented by an MHC antigen to a T lymphocyte by an MHC-bearing antigen presenting cell. The MHC molecule may be DQw2 or any other histocompatibility antigen that functions to present antigen to T

lymphocytes. The invention is specifically directed to the use of such a peptide in the subset of patients bearing clonal expansions of T cells containing receptors with the Vα12.1 rearrangement. Thus, the invention is directed to all of the hereinbefore described methods wherein the peptide comprises an amino acid sequence I derived from the Vα12.1 rearrangement.

It is to be understood, however, that treatment with the peptides of the present invention that are derived from DQw2 is not restricted to patients with rheumatoid arthritis who bear the Vα12.1 clonal expansion. It is understood that the presentation of DQw2 as an antigen to T lymphocytes may occur wherein the T lymphocyte does not necessarily bear the Vα12.1 rearrangement but where the DQw2, recognized by a T lymphocyte as nonself, may be recognized by a T lymphocyte other than the lymphocyte with the Vα12.1 rearrangement such that said lymphocyte becomes clonally expanded.

The invention is also generally directed to methods using antibody reagents wherein said antibodies are developed against the DQw2 protein itself. Accordingly, the invention is directed to a method for preventing antigen presentation in patients with rheumatoid arthritis, which patients express the MHC HLA DQw2 allele, comprising administering an antibody against the MHC HLA DQw2 protein, which is effective in blocking the interaction of a DQw2-bearing antigen-presenting cell with a T lymphocyte. Thus, the present invention is directed to a method for preventing T lymphocyte activation by use of the above method, a method for preventing or ameliorating a chronic inflammatory response in patients with rheumatoid arthritis and expressing the MHC HLA DQw2 allele, which response results from the persistent activation of T lymphocytes by an MHC-bearing antigen-presenting cell, by the application of the above method, and more generally, a method for treating rheumatoid arthritis in these patients.

It is to be understood that the antibody may be used to bind the DQw2 molecule when it functions as an antigen-bearing MHC found on an antigen-presenting cell or may be developed against a DQw2 peptide which functions as the *presented* antigen. In the first case, the DQw2 functions to present a

peptide derived from the processing of an endogenous protein and which is recognized by the T-lymphocytes as foreign. In this case, the antibody may be directed against any of those portions of DQw2 which, when bound by antibody, prevent an interaction with a relevant T lymphocyte, which interaction is necessary for the subsequent activation of that T lymphocyte. When the antibody is developed against the specific region of DQw2 which is presented as an antigen, the antibody likewise functions to prevent the interaction of the relevant T lymphocyte with the DQw2 peptide which has become recognized as a foreign antigen. In this case, the DQw2 peptide is processed by an MHC molecule on an antigen-presenting cell. This MHC molecule may be either DQw2 itself or may be one of the other histo-compatibility antigens which functions in presenting a relevant antigen to a T lymphocyte for a normal immune response.

The above methods may be practiced in patients bearing a clonal expansion of a T lymphocyte expressing the variable α chain rearrangement Vα12.1 protein. However, the methods are not restricted to patients bearing T cells with this rearrangement. Thus, the methods are practiced in any patient with rheumatoid arthritis, which patient expresses the DQw2 haplotype.

The invention also encompasses administration of the antibody of the present invention to patients wherein the processed peptide which is recognized as nonself may be any of a variety of autoantigens other than DQw2 and Vα12.1. In these embodiments, for the purposes of the invention, this autoantigen is presented by an antigen-presenting cell bearing the protein gene product of the DQw2 locus.

The methods of the present invention may be practiced either with polyclonal or monoclonal antibodies.

As used herein, "treatment" is meant to include both prophylactic treatment to prevent an autoimmune disease having the symptoms of rheumatoid arthritis (or the manifestation of clinical symptoms thereof) as well as the therapeutic treatment, i.e., the suppression or any measurable alleviation

of one or more symptoms after the onset of a disease presenting the symptoms of rheumatoid arthritis.

By the term "autoantigen" is intended for the purposes of the invention any substance normally found within a mammal that, in an abnormal situation, is no longer recognized as part of the mammal itself by the lymphocytes or antibodies of that mammal, and is therefore attacked by the immunoregulatory system as though it were a foreign substance.

By the term "MHC" or "major histocompatibility complex" is meant a complex series of mammalian cell surface proteins present on the surface of activated T cells, macrophages and other immune cells. The MHC plays a central role in many aspects of immunity, both in presenting histocompatibility (or transplantation) antigens and in regulating the immune response against conventional (foreign) antigens. The human MHC genes are located on human chromosome 6 and the mouse MHC genes are located in the H-2 genetic locus on mouse chromosome 17. For the purposes of the present invention, the relevant MHC expression products are those that present DQw2 peptide in rheumatoid arthritis patients and in the DQw2 expression product as an antigen-presenting MHC molecule.

"Class II MHC molecules" are membrane glycoproteins that form part of the MHC. Class II MHC molecules are found mainly on cells of the immune system, including B cells, macrophages, brain astrocytes, epidermal Langerhans's cells, dendritic cells, thymic epithelium, and helper T cells. Class II MHC molecules are involved in regulating the immune response during tissue graft rejection, stimulation of antibody production, graft versus host reactions, and in the recognition of self (or autologous) antigens, among other phenomena.

By the term "T cells" or "T lymphocytes" is meant immune system cells derived from stem cells located within hematopoietic (i.e., blood forming) tissues. There are three broad categories of T cells: helper, suppressor, and cytotoxic. T cells express either the CD4 antigen (and are then called CD4+ T cells) or the CD8 antigen (in which case they are called

CD8+ T cells) on their cell surface. The expression of CD4 and CD8 antigens by peripheral (circulating) T cells correlates with the function and specificity of the T cell.

By the term "T cell receptor" or "TCR" is meant the antigen recognition receptor present on the surface of T cells. TCR is, therefore, the receptor that binds a molecule which the immune system recognizes—and presents—as an antigen (whether the molecule is foreign or autologous, the latter being the case in an autoimmune disease). A majority of T cells express a TCR composed of a disulfide bond and heterodimer protein containing one α and one β chain, whereas a minority of T cells express two different chains (γ and δ). The TCR is composed of an α and a β chain, each of which comprises a variable and a constant region. The variable in turn comprises a variable, a diversity, and a joining segment. The junction among the variable, diversity and joining segment is postulated to be the site of antigen recognition by T cells.

T cells initiate the immune response when antigen presenting cells (APC), such as mononuclear phagocytes (macrophages, monocytes), Langerhans's cells and follicular dendritic cells, initially take up, process (digest) and present antigenic fragments of their polypeptide on their cell surface (in connection with their MHC). CD4+ T cells recognize antigen molecules exclusively when the protein is processed and peptide fragments thereof are presented by APCs that express class II MHC molecules.

T cell recognition of an antigen reflects a trimolecular interaction between the TCR, MHC molecules and peptides processed by APCs via a cleft or pocket in the three-dimensional structure of the class II MHC molecule (Bjorkman, P.J., *et al.*, *Nature 329*:506 and *Nature 329*:512 (1987)).

For the purposes of the present invention, the T cell receptor relevant to the present invention may be, but is not limited to, receptor expressing the Vα12.1 α variable region rearrangement. The T cell receptor of interest to the present invention is clonally expanded in a subset of rheumatoid arthritis patients. Accordingly, the present invention is relevant to clonally expanded

populations of T cells, identified by sequencing of T cell receptor cDNA, according to the exemplary material herein, which clonal expansion has resulted from interaction with an autologous antigen (autoantigen).

By the term "MHC HLA DQw2", "HLA DQw2 or "DQw2" is intended that allele of a protein encoded by the DQ region in the major histocompatibility complex of genes according to Figure 1 herein. Three alleles at the DQ locus are currently known to exist. Of these three polymorphic alleles, the second has been tentatively designated as DQw2.

By the term "amino acid sequence (I)" is intended a sequence analog, which sequence is analogous to a sequence derived from a naturally occurring protein. The amino acid sequence (I) may be from about 8-24 amino acids in length. The significance of this amino acid sequence is that it is an analog of an amino acid sequence normally found in the body and which is presented by the MHC to a T lymphocyte. For the specific purposes of the invention, the amino acid sequence is analogous to that sequence in rheumatoid arthritis patients which is presented by MHC and is responsible for the T cell inflammatory response that is associated with rheumatoid arthritis.

By "amino acid sequence II" is intended the naturally occurring peptide that is presented to a T lymphocyte in rheumatoid arthritis patients and which is presented to T lymphocytes in such patients and is responsible for the inflammatory response characteristic of rheumatoid arthritis. In rheumatoid arthritis patients bearing the DQw2 MHC haplotype, amino acid sequence II is that portion of the DQw2 protein sequence which is presented to the T lymphocyte by the MHC. In rheumatoid arthritis patients with a clonal expansion of a T lymphocyte bearing a specific T cell receptor rearrangement, particularly the Vα12.1 rearrangement, amino acid sequence II is that portion of the receptor sequence which is presented to a T lymphocyte by an MHC antigen, particularly the DQw2 MHC antigen.

By "analog" or "analogous" is intended for the purpose of the invention an amino acid sequence which is similar to a second amino acid sequence, but which alters the function of the first amino acid sequence. For example, a

variant of DQw2, as the antigen being presented, is altered in sequence such that it will bind to the MHC but will prevent the activation of the T lymphocyte recognizing the true DQw2 sequence either by preventing binding to the T cell receptor or, while not preventing binding, preventing subsequent steps that lead to T cell activation. In this manner, the T lymphocyte which normally recognizes DQw2 as a foreign antigen is inhibited from activation and subsequent expansion and cytotoxic effects.

Preparation of Antibodies

Antibodies may be prepared against soluble DQw2, DQw2 complexed with antigen, or DQw2 complexed with antigen as part of an antigen presenting cell/MHC/antigen complex. Alternatively, antibodies may be prepared against DQw2 peptides complexed with reagents rendering said peptides immunogenic. The amino acid sequence of the DQw2 allele is known in the art (see Figure 3, Wu *et al.*, *Human Immunol. 27*:305-322 (1990), which is incorporated herein by reference).

Antibodies may be prepared in accordance with conventional techniques, particularly employing the monoclonal antibody techniques as described, for example, in U.S. Patent Nos. 4,690,893; 4,713,325; 4,714,681; 4,716,111; and 4,720,459.

Any of a number of techniques may be employed for identifying the presence of a T cell receptor binding to the particular monoclonal antibody or antiserum. A wide variety of labels have been used for detection, such as particles, enzymes, chromophors, fluorophors, chemiluminescents, and the like. Any particular label or technique which is employed is not critical to this invention, and any convenient technique may be employed. The techniques may either be competitive or non-competitive methodologies, including sandwich methodologies. The cells will usually be lysed to provide membrane-free proteins in accordance with conventional techniques. Cellular debris may be removed and the protein extracted and harvested.

Alternatively, intact cells may be employed and detected by fluorescence activated cell sorting or the like.

For therapeutic purposes, there may be an interest in using human antibodies. Normally, one would not be permitted to immunize a human host with the protein or fragment of interest to activate T cells specific for the sequence of interest. However, there are alternatives, in that mice or other lower mammals may be immunized, and the genes encoding the variable regions of the antibodies specific for the region of interest isolated and manipulated by joining to an appropriate human constant region, and optionally, the complementarity determining regions used to replace the complementarity determining regions of a human antibody by genetic engineering. The resulting chimeric construct comprising a lower mammalian variable region or CDR and a human constant region may then be removed into a microorganism or mammalian host cell in culture, particularly a lymphocyte, and the hybrid antibodies expressed. In some instances it may be satisfactory to use mouse antibodies where tolerance can be achieved or some degree of immune suppression may be involved.

Either the entire antibody may be administered, or Fab fragments, or even only the Fv region. By removing all or a portion of the constant region, there may be a reduction in the immune response. For therapeutic purposes, the antibody may be formulated with conventional pharmaceutically or pharmacologically acceptable vehicles for administration, conveniently by injection. Vehicles include deionized water, saline, phosphate buffered saline, Ringer's solution, dextrose solution, Hank's solution, etc. Other additives may include additives to provide isotonicity, buffers, preservatives, and the like. The antibody may be administered parenterally, typically intravenous or intramuscularly, as a bolus, intermittently or in a continuous regimen. Typical doses for adult humans will be in the range of about 1 ng-100 mg/kg body weight. A preferred dosage is between about 10 ng and 10 mg/kg, and a more preferred range is between 100 ng and 1 mg/kg. Doses for children or

other animal species may be extrapolated from the adult human doses based on relative body weight.

Synthesis of Peptides

A random set of overlapping peptides is synthesized from the variable domain of Vα12.1. Regarding the synthesis of DQw2 peptides, random sets of overlapping peptides are synthesized from each of the following domains: α1, α2, β1, and β2. In order to identify the sequence of peptides, hitherto unknown, which are presented by DQw2, such peptides are identified by releasing them from DQw2 cells of rheumatoid arthritis patients such as by acid elution or other denaturing measures as used for release of class I peptides. The peptides are separated by high pressure liquid chromatography and subjected to microsequencing for determination of the amino acid sequence. These procedures are known to those of ordinary skill in the art. For example, see Rudensky *et al.*, *Nature 353*:622 (1991).

The peptide sequence to be synthesized is part of a sequence of an immunogen of interest associated with an autoimmune disease. Regarding the instant application, the peptide sequence is that sequence which is presented by a major histocompatibility antigen to a T lymphocyte in order to induce activation of that T lymphocyte. The oligonucleotide comprising the subject peptide may be from any site of the immunogen sequence, that is, N terminal or C terminal proximal or central, where the oligopeptide sequence will normally be substantially homologous with from about 8-24 amino acids of the immunogen sequence, although longer sequences may also be employed. Usually, the difference in homology between a natural sequence and the oligopeptide which is employed will be not more than three lesions, usually not more than one lesion, which may be insertions, deletions, or conservative or non-conservative substitutions.

The compositions of this invention will include usually at least one sequence of an immunogen of interest, including the subject peptide, and may

include two or more oligopeptide sequences containing sequences of from about 8-24 amino acids present in the immunogen, depending on the number of presented amino acid sequences present in the immunogen. Thus, if there are a plurality of these sequences present in the immunogen, all or fewer than all of the sequences may be employed in a single composition.

In preparing the subject compositions, one would select an immunogen related to the particular manifestation of autoimmunity in a patient. For example, in a patient with the Vα12.1 rearrangement and clonal expansion, the immunogen would be selected from this protein product. Similarly, in DQw2 positive rheumatoid arthritis patients, the immunogen would be selected from the DQw2 molecule. Alternatively, in DQw2 positive rheumatoid arthritis patients with a clonal expansion of a T lymphocyte containing a specific T cell receptor rearrangement, the immunogen would be said T cell receptor chain containing said rearrangement. Alternatively, the immunogen of interest may be a peptide in DQw2 positive or Vα12.1 positive patients which is other than these two proteins, but which is presented by the DQw2 MHC or other MHC to Vα12.1 positive T lymphocytes or other lymphocytes.

The subject oligopeptides may be administered in a variety of ways, by themselves or in conjunction with various additives. Various carriers may be employed which are physiologically acceptable such as water, alcohol, saline, phosphate buffered saline, sugar, mineral oil, etc. Other additives may also be included as stabilizers, detergents, flavoring agents, thickeners, etc. The amount of active ingredient administered will vary widely depending upon the particular composition, the particular host, the number and frequency of administrations, the manner of administration, age, health, etc.

Peptides based on the sequence of DQw2, Vα12.1, or other clonally expanded T cell rearrangements and autologous peptides causing such clonally expanded T cells in rheumatoid arthritis patients can be synthesized using well known solid phase methods (Merrifield, R.B., *Fed. Proc. Am. Soc. Exp. Biol.* *21*:412 (1962) and *J. Am. Chem. Soc.* *85*:2149 (1963); Mitchel, A.R., *et al.*, *J. Am. Chem. Soc.* *98*:7357 (1976); Tam, J., *et al.*, *J. Am. Chem. Soc.*

105:6442 (1983)). Alternatively, such peptides can be synthesized by recombinant DNA techniques, as is now well known in the art (Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, New York (1982), see pp. 51-54 and pp. 412-30). For example, these peptides can be obtained as the expression products after incorporation of DNA sequences encoding the immunogen (or fragments or analogs thereof) into expression vectors and introduction of such vectors into suitable eukaryotic or prokaryotic hosts that will express the desired peptides individually or as part of fusion peptides or proteins.

Peptide analogs can be designed using the known amino acid sequences encoded by the genes of interest as disclosed below, using the synthetic or recombinant techniques described above and the methods of, e.g., Eyler, E.H., in *Adv. Exp. Med. Biol. 98*:259-281 (1978). For example, a peptide having a sequence based upon the amino acid sequence of DQw2 or Vα12.1 can be chemically synthesized using the above-described techniques. The peptide can be tested for disease-suppressive activity when administered to a mammal using, for example, the experimental protocol of Howell, M.D., *et al.*, *Science 246*:668 (1989) or Vanderbark, A.A., *et al.*, *Nahire 341*:541 (1989).

Alternatively, amino acid sequence variants of the peptide can be prepared by mutations in the DNA which encodes the synthesized peptide. Such variants include, for example, deletions from, or insertions and substitutions of, residues within the amino acid sequence. Any combination of deletion, insertion, and substitution may also be made to arrive at the final construct, provided that the final construct possesses the desired activity.

At the genetic level, these variants ordinarily are prepared by site-directed mutagenesis of nucleotides in the DNA encoding the peptide molecule, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. The variants typically exhibit the same qualitative biological activity as the nonvariant peptide.

Preparation of a peptide variant in accordance herewith is preferably achieved by site-specific mutagenesis of DNA that encodes an earlier prepared variant or a nonvariant version of the relevant protein or peptide. Site-specific mutagenesis allows the production of peptide variants through the use of specific oligonucleotide sequences that encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. The technique of site-specific mutagenesis is well known in the art, as exemplified by Adelman *et al.*, *DNA 2*:183 (1983). Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage, for example, as disclosed by Messing *et al.*, *Third Cleveland Symposium on Macromolecules and Recombinant DNA*, Walton, A., ed., Elsevier, Amsterdam (1981). These phages are readily commercially available and their use is generally well known to those skilled in the art. Alternatively, plasmid vectors that contain a single-stranded phage origin of replication (Veira *et al.*, *Meth. Enzymol. 153*:3 (1987)) may be employed to obtain single-stranded DNA.

In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector that includes within its sequence a DNA sequence that encodes the relevant peptide. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically, for example, by the method of Crea *et al.*, *Proc. Natl. Acad. Sci. (USA) 75*:5765 (1978). This primer is then annealed with the single-stranded protein-sequence-containing vector, and subjected to DNA-polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, to complete the synthesis of the mutation-bearing strand. Thus, a mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells and clones are selected that include recombinant vectors bearing the mutated sequence arrangement. The mutated protein region may be removed and placed in an appropriate vector

for protein production, generally an expression vector of the type that may be employed for transformation of an appropriate host.

An example of a terminal insertion includes a fusion of a signal sequence, whether heterologous or homologous to the host cell, to the N-terminus of the peptide molecule to facilitate the secretion of mature peptide molecule from recombinant hosts.

Another group of variants are those in which at least one amino acid residue in the peptide molecule, and preferably only one, has been removed and a different residue inserted in its place. Such substitutions preferably are made in accordance with the following list when it is desired to modulate finely the characteristics of a peptide molecule.

Original Residue	Exemplary Substitutions	Original Residue	Exemplary Substitutions
Ala	gly; ser	Leu	ile; val
Arg	lys	Lys	arg; gln; glu
Asn	gln; his	Met	leu; tyr; ile
Asp	glu	Phe	met; leu; tyr
Cys	ser	Ser	thr
Gln	asn	Thr	ser
Glu	asp	Trp	tyr
Gly	ala; pro	Tyr	trp; phe
His	asn; gln	Val	ile; leu
Ile	leu; val		

Substantial changes in functional or immunological properties are made by selecting substitutions that are less conservative that those in the above list, that is, by selecting residues that differ more significantly in their effect on maintaining (a) the structure of the peptide backbone in the area of the substitution, for example, as a sheet or helical conformation; (b) the charge or hydrophobicity of the molecule at the target site; or (c) the bulk of the side chain. The substitutions that in general are expected to those in which (a) glycine and/or proline is substituted by another amino acid or is deleted or inserted; (b) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl, or alanyl; (c) a cysteine residue is substituted for (or by) any other residue; (d)

a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) a residue having an electronegative charge, e.g., glutamyl or aspartyl; or (e) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having such a side chain, e.g., glycine.

Most deletions and insertions, and substitutions in particular, are not expected to produce radical changes in the characteristics of the peptide molecule. However, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays. For example, a variant typically is made by site-specific mutagenesis of the peptide molecule-encoding nucleic acid, expression of the variant nucleic acid in recombinant cell culture, and, optionally, purification from the cell culture, for example, by immunoaffinity adsorption on an anti-peptide antibody column (to absorb the variant by binding it to at least one epitope).

Example 1

The relative percentage of TCR Vα12.1$^+$ T cells in rheumatoid arthritis patients was assessed using Vα12.1-specific mAb 6D6 in both CD4$^+$ and CD8$^+$ subsets by two-color staining and flow cytometry. One group of patients (Vα12.1-normal) contained percentages of TCR Vα12.1$^+$, CD8$^+$ T cells (mean of 3.6%, range 1.0% to 7.0%) which was similar to those found in normal subjects (Figure 2) (Der Simonian *et al.*, *J. Exp. Med. 174*:639 (1991)). However, the distribution was bimodal, as a second group of patients (Vα12.1-elevated) had much higher percentages of Vα12.1$^+$ T cells (mean value of 22%, range 8.0% to 43%) in the CD8$^+$ subset. This group of patients contained greater than 7.5% of Vα12.1 bearing CD8$^+$ T cells (above two standard deviations (7.3%) from the mean (3.6%) of Vα12.1-normal and healthy controls). Each of the nine patients in this Vα12.1-elevated group, contained 43%, 28%, 26%, 22%, 21%, 20%, 18%, 12%, 10% and 8.5%

Vα12.1 bearing CD8$^+$ T cells, respectively. The high percentages of Vα12.1$^+$ T cells in patients was a relatively stable phenomenon as tested over one or two year period showed consistent levels. For instance, the Vα12.1 percentage in patient #3 (MG) was 26.5% (10/89), 22.5% (5/91), and 28.5% (6/91) and for patient #4 (AL) was 26.5% (6/91) and 19% (11/91). To gain insight into the basis for the Vα12.1$^+$ T cell expansion in rheumatoid arthritis, Vα12.1 transcripts from positively selected CD8$^+$T cells were cloned and sequenced. In each of the three patients analyzed, distinct, repeated Vα12.1 containing sequences corresponding to functional TCR a-chain transcripts were identified. For example, in patient 1 where 43% of the CD8$^+$T cells were Vα12.1$^+$, all 15 DNA clones analyzed had identical sequences. Similarly, 9 of 16 (56%) Vα12.1 containing DNA clones were identical in patient #2, where 26% of COB' T cells were Vα12.1$^+$. In patient 3, where 28% of CD8$^+$ T cells were Vα12.1$^+$, 2 distinct sequences which were repeated were identified. One sequence was represented in 16 of 23 DNA clones (70%), and a second repeated sequence was identified in 4 of 23 DNA clones (17%). The remaining 3 clones sequenced were represented only once. Although the junctional sequences were different, these two Vα12.1 encoded sequences used the same JαA6 gene segment (Figure 3). Furthermore, by a second independent method using inverse PCR (iPCR), Vα containing transcripts from CD4-depleted and Vα12.1$^+$ selected T cells were cloned and sequenced. This permitted the amplification of all TCR Vα transcripts present in Vα12.1$^+$/CD8 selected T cells. As predicted by the direct PCR method, 9 of 9 α-chain DNA clones in patient 1 were identical, but distinct from a repeated sequence found in 21 of 27 DNA clones (78%) from patient 3. These sequences match exactly the repeated sequences generated by the direct PCR method, respectively. Moreover, the second clonal population of Vα12.1 to JαA6 recombination as seen in patient 3 (by direct PCR method) was also independently confirmed by the iPCR method (Uematsu *et al.*, *Proc. Natl. Acad. Sci. USA* 88:8534 (1991)), where 5 of 27 DNA clones (19%) were found to be identical. Thus, two independent

methods of generating TCR Vα specific PCR products validate each approach and confirm the oligoclonality of Vα12.1 expansion in rheumatoid arthritis.

Notably, all of the repeated Vα12.1$^+$ T cell rearrangements in the 3 patients analyzed use either JαAl (patient #1), JαA12 (patient #2) or JαA6 (patient #3) each of which encodes a unique sequence at the 3′ end of Jα gene segment. This short stretch of shared residues (pro-tvr) is predicted to contribute (or is immediately adjacent) to the third complementarity determining region (CDR3) and thus may play a role in antigen or MHC recognition. Interestingly, only six of the 80 known Jα gene segments encode this two amino acid sequence stretch. The striking occurrence of repeated sequences found in these patients was not observed in similar analysis of normal subjects (data not shown), and it suggested a corresponding clonality of Vα12.1$^+$ T cells in the patient's peripheral blood. To obtain further information on the apparently clonal nature of the Vα12.1$^+$/CD8$^+$ peripheral T cells in rheumatoid arthritis patients, α-chain transcripts were analyzed using the iPCR to study all of the Vβs associated in Vα12.1$^+$/CD8$^+$ T cells (see Figure 3 legend). All TCR Vα transcripts present in the Vα12.1$^+$/CD8$^+$ T cells were amplified. In patient 1, 18 of 18 Vβ containing clones were identical. Similarly, in patient 2, 12 of 20 were identical, but these were different Vβ segments. For instance, patient 1 used Vβ5.1 rearranged to Dβ1.1/β2.7, while in patient 2, VβB was rearranged to Dβ1.1/Jβ.2, respectively (Figure 3B). In order to confirm the cell surface co-expression of Vα12.1 and Vβ8 on the peripheral CD8$^+$ T cells in patient 3, two-color staining was done on CD4 depleted PBL using Vαl2.1 and Vβ8 specific mAb, 16GB. Almost 75% of Vα12.1$^+$ T cells were co-stained with the Vβ8-specific mAb (Figure 3C), confirming the functional co-expression of the same gene segments whose sequences were observed by molecular cloning.

The surface expression of several activation markers, including IL-2R, HLA-DR, and CD45RO, were analyzed by FACS (Figure 4). Similar to the total CD8$^+$T cell staining profile, Vα12.1$^+$ T cells expressed mostly IL-2R β-chain. No significant number of freshly isolated Vα12.1$^+$ T lymphocytes

expressed the "high affinity" IL-2R ad-chains. The expression of transferrin receptor on Vα12.1$^+$ T cells also was analyzed. However, no detectable levels were found. CD45RO was expressed on majority of the Vα12.1$^+$ T cells indicating a memory phenotype. These results suggested that the majority of the Vα12.1$^+$ cells in circulation at least in the 3 individuals analyzed were not acutely activated but may have been previously stimulated. Further support for this hypothesis comes from the substantial Vα12.1$^+$ T cells (10% to 30%) expressed HLA-1 and HLA-DR, suggesting ongoing activation for a fraction of these cells.

Further, MHC alleles present in the Vα12.1-elevated group were examined and compared to the group of rheumatoid arthritis patients lacking expansion of Vα12.1 bearing T cells (Figure 5). Both the Vα12.1-elevated and the Vα12.1-normal group expressed HLA-DR1 and -DR4 alleles as expected for adult rheumatoid arthritis patients. However, among the Vα12.1-elevated group of rheumatoid arthritis patients, the frequency of HLA DQw2 was increased as it was found in 6 of 7 (86%) of the patients (Figure 5). In general, the frequency for the DQw2 (DQB/0201) encoding allele, linked with DR3-(DQAl/0501) and DR7-(DQAl/020l) extended haplotype, is approximately one in three (depending on the ethnic background), and a little less than 1 in 3 of the Vα12.1-normal group were DQw2 positive. However, when all of the Vα12-elevated individuals identified so far including nor-rheumatoid arthritis patients were studied, the association of HLA-DQw2 in individuals with elevated Vα12.1$^+$/CD8$^+$ T cells is highly significant ($p = 0.001$).

Here evidence is provided for the expansion of clonal populations of T cells in rheumatoid arthritis that were encoded by Vα12.1 gene segment rearrangement to J11 genes containing the pro-tvr dipeptide. These populations were readily detected using the simple screening technique of mAb staining and flow cytometry, which allowed examination of a relatively large number of subjects. The expansions found were present in approximately 1 of 6 (19%) patients and only in the CD811+ population of (peripheral blood)

T cells. It is widely assumed that the clinical diagnosis of rheumatoid arthritis may include at least several specific diseases. Thus it is suggested that the Vα12.1-elevated group of rheumatoid arthritis patients represents a subset defined by an immunologic aberration, the expansion of Vα12.1 bearing CDB T cells and the increased association of the HLA DQw2 haplotype. This group of patients, however, did not differ from the Vα12.1-normal group of R3 patients in their clinical parameters (such as ESR, PCV).

The mechanism responsible for the clonal Vα12.1$^+$ T cell expansion in this subset of rheumatoid arthritis patients is not known. However, the restricted Vα/Jα usage in unrelated patients and CD45RO expression by the cells provide support for a highly specific antigen driven expansion. Several examples of restricted Vβ, vα and Jα usage have been reported to characterize the T cell response to specific foreign antigen in the setting of an MHC background such as cytochrome c (Winoto *et al.*, *Nature 324*:679 (1986); Sorger *et al.*, *J. Exp. Med. 165*:279 (1987); lambda (I) repressor cI protein 8 and LCMV glycoprotein. Similarly restricted germline usage has been noted in autoimmune animal models such as EAE in response to MOP. It is possible that a marked or persisting response may eventually lead to the proliferation of selected antigen activated T cell clones that ultimately dominate as clonal population of circulating cells. The high frequency of HLA DQw2 in particular in the Vα12.1-elevated group of patients implicates this class II molecule in the process. However, it is not known if the expanded CDB' T cells are MHC class II (DQw2) restricted or possibly reactive to DQw2 derived peptides (or other class II peptides) recognized in the context of MHC class I molecules. Interestingly, a role for HLA-00 molecules in the expansion of COB' T cells may be important in autoimmune regulation or immunoglobulin production as demonstrated in a different system by Salgame *et al.*, *Proc. Natl. Acad. Sci. USA 88*:2598 (1991). Whether the Vα12.1$^+$ T cell population or the antigen to which it responds are critical to the process of rheumatoid arthritis remains to be determined. However, this finding of striking clonal expansions that account for one fifth to nearly one half of the

CD8 T cells in the periphery of a group of patients with rheumatoid arthritis is highly relevant to the diagnosis of rheumatoid arthritis and yields insight into the pathophysiology of the disease process.

Having now generally described the invention, the various modifications and uses that are encompassed therein will be evident to the person of ordinary skill in the art.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: Reagents Useful In Treatment of Rheumatoid Arthritis

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGGTCGACGA CCTCATGTCT AGCACAGT 28

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GCATGCGGCC GCCCTGCTAT GCTGTGTGTC T 31

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both

(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGGTCGACAC ACAGCGACCT CGGCTGGG 28

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCATGCGGCC GCCATGGTCA AGAGAAAGGA 30

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asp Gly Tyr Gly Gln Asn Phe Val Phe Gly Pro Gly Thr Arg Leu Ser
1 5 10 15

Val Leu Pro Tyr
20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Asp Tyr Gln Gly Gly Ser Glu Lys Leu Val Phe Gly Lys Gly Met Lys
1 5 10 15

Leu Thr Val Asn Pro Tyr
20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gly Ala Tyr Asn Thr Asn Ala Gly Lys Ser Thr Phe Gly Asp Gly Thr
1 5 10 15

Thr Leu Thr Val Lys Pro Asn
20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Thr Gln Thr Asn Ala Gly Lys Ser Thr Phe Gly Asp Gly Thr Thr Leu
1 5 10 15

Thr Val Lys Pro Asn
20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Val Gly Gly Ala Asn Asn Leu Phe Phe Gly Thr Gly Thr Arg Leu Thr
1 5 10 15

Val Ile Pro Tyr
20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Pro Gly Ser Asn Asn Asp Met Arg Phe Gly Ala Gly Thr Arg Leu Thr
1 5 10 15

Val Lys Pro Asn
20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gly Ile Asp Asp Lys Ile Ile Phe Gly Lys Gly Thr Arg Leu His Ile
1 5 10 15

Leu Pro Asn

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Leu Arg Ser Gly Gly Tyr Asn Lys Leu Ile Phe Gly Ala Gly Thr Arg
1 5 10 15

Leu Ala Val His Pro Tyr
20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Trp Gly Gly Ser Gln Gly Asn Leu Ile Phe Gly Lys Gly Thr Lys Leu
1 5 10 15

Ser Val Lys Pro Asn
20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Arg Gly Gly Gly Ala Asp Gly Leu Thr Phe Gly Lys Gly Thr His Leu
1 5 10 15

Ile Ile Gln Pro Tyr
20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Pro Tyr Ser Gly Gly Gly Ala Asp Gly Leu Thr Phe Gly Lys Gly Thr
1 5 10 15

His Leu Ile Ile Gln Pro Tyr
20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Pro Gly Val Thr Asp Ser Asn Tyr Gln Leu Ile Trp Gly Ala Gly Thr

1 5 10 15

Lys Leu Ile Ile Lys Pro Asn
20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Pro Asn Thr Gly Arg Arg Ala Leu Thr Phe Gly Ser Gly Thr Arg Leu
1 5 10 15

Gln Val Gln Pro Asn
20

What Is Claimed Is:

1. A peptide comprising an amino acid sequence(I) that is analogous to an amino acid sequence(II) that is presented to a T lymphocyte, in rheumatoid arthritis patients, by an MHC protein-bearing antigen-presenting cell and wherein said amino acid sequence(II) is derived from DQw2.

2. A peptide comprising an amino acid sequence(I) that is analogous to an amino acid sequence(II) that is presented to a T lymphocyte, in rheumatoid arthritis patients, by an MHC protein-bearing antigen-presenting cell, wherein said amino acid sequence(II) is derived from V alpha 12.1 and wherein said patients express a T-cell receptor with a V alpha 12.1 rearrangement.

3. The peptide of either of claims 1 or 2 wherein said MHC protein is MHC HLA DQw2.

4. A peptide sequence presented *in vivo* in rheumatoid arthritis patients by MHC HLA DQw2 on an antigen-presenting cell, that stimulates T lymphocytes bearing Vα12.1 T cell receptor rearrangement.

5. A method for preventing T cell activation in patients with rheumatoid arthritis, said patients expressing the MHC HLA DQw2 allele, comprising administering the peptide of any of claims 1-4 in an amount sufficient to prevent said activation.

6. A method for preventing antigen presentation in patients with rheumatoid arthritis, said patients expressing the MHC HLA DQw2 allele, comprising administering the peptide of any of claims 1-4 in an amount sufficient to prevent said presentation.

7. A method for preventing antigen presentation in patients with rheumatoid arthritis, said patients expressing a T cell receptor bearing the V alpha 12.1 rearrangement, comprising administering the peptide of either of claims 2 or 4 in an amount sufficient to prevent said presentation.

8. A method for preventing antigen presentation in patients with rheumatoid arthritis, said patients expressing the MHC HLA DQw2 allele, comprising administering an antibody against the MHC HLA DQw2 protein, said antibody being effective in blocking the interaction of a DQw2-bearing antigen-presenting cell with a T lymphocyte.

9. A method for preventing T lymphocyte activation by an antigen in patients with rheumatoid arthritis, said patients expressing the MHC HLA DQw2 allele, comprising administering an antibody against the MHC HLA DQw2 protein, said antibody being effective in preventing T lymphocyte activation by interaction of said T lymphocyte with a DQw2-bearing antigen-presenting cell.

10. A method for preventing or ameliorating a chronic inflammatory response in patients with rheumatoid arthritis and expressing the MHC HLA DQw2 allele, said response resulting from the persistent activation of T lymphocytes by a DQw2-bearing antigen-presenting cell, said method comprising administering an antibody against the MHC HLA DQw2 protein, said antibody being effective in preventing the chronic inflammation caused by persistent T lymphocyte activation by interaction of said T lymphocyte with a DQw2-bearing antigen-presenting cell.

11. The method of any of claims 8, 9, or 10 wherein said antibody is a monoclonal antibody.

12. The method of any of claims 8, 9, or 10 wherein said T lymphocyte expresses the variable alpha chain rearrangement V alpha 12.1 protein.

13. The method of any of claims 8, 9, or 10 wherein said antigen presenting cell is selected from the group consisting of a macrophgage, monocyte, dendritic cell, Langerhan's cell, and B cell.

14. The method of any of claims 8, 9, or 10 wherein said antigen is an autoantigen.

15. The method of claim 14 wherein said autoantigen is selected from the group consisting of DQw2 and V alpha 12.1.

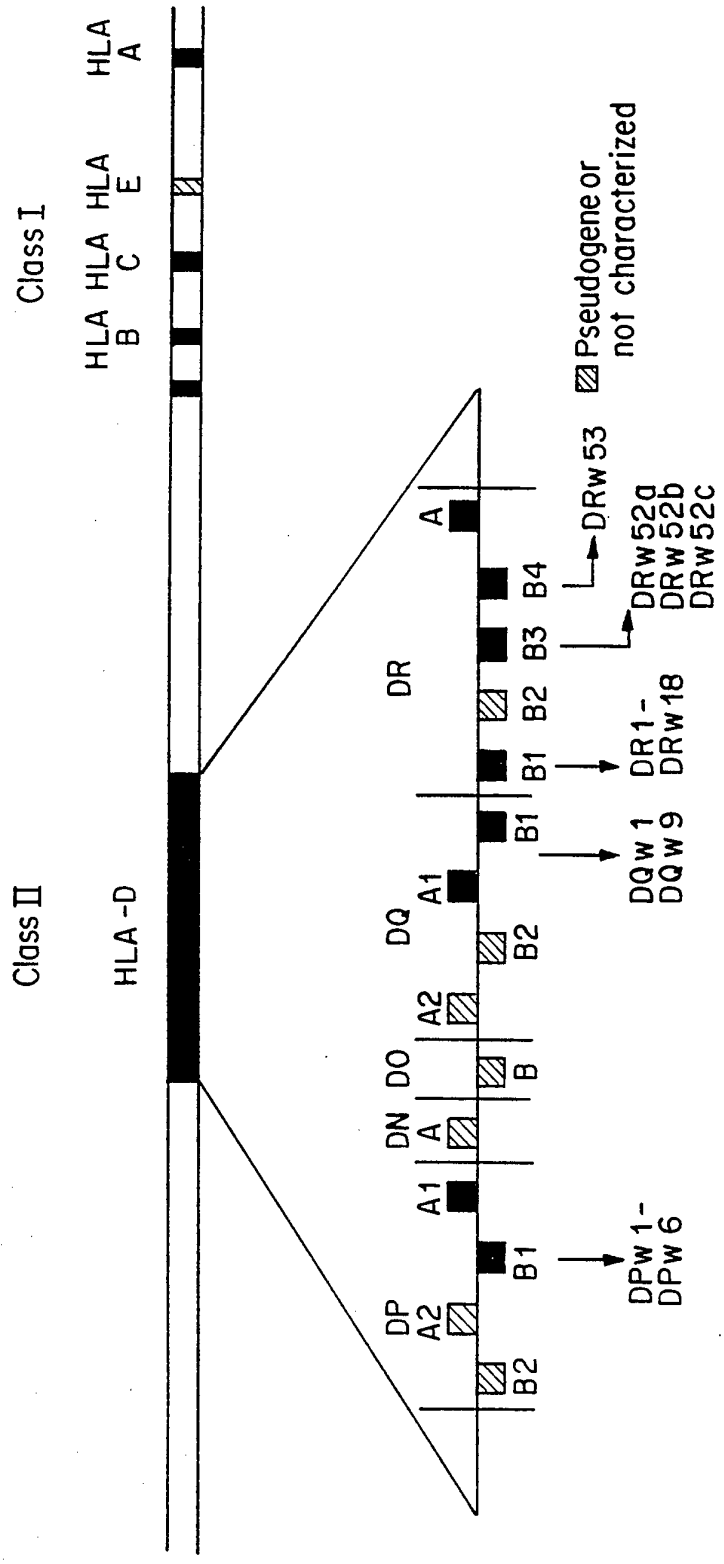

Class I
HLA A
HLA HLA E
HLA HLA C
HLA B
Class II
HLA-D
DR
DQ
DO
DN
DP
A
B1
B2
B3
B4
A1
A2
B
DRw 53
DRw 52a
DRw 52b
DRw 52c
DR1-
DRw 18
DQw 1
DQw 9
DPw 1-
DPw 6
Pseudogene or
not characterized

FIG. 1

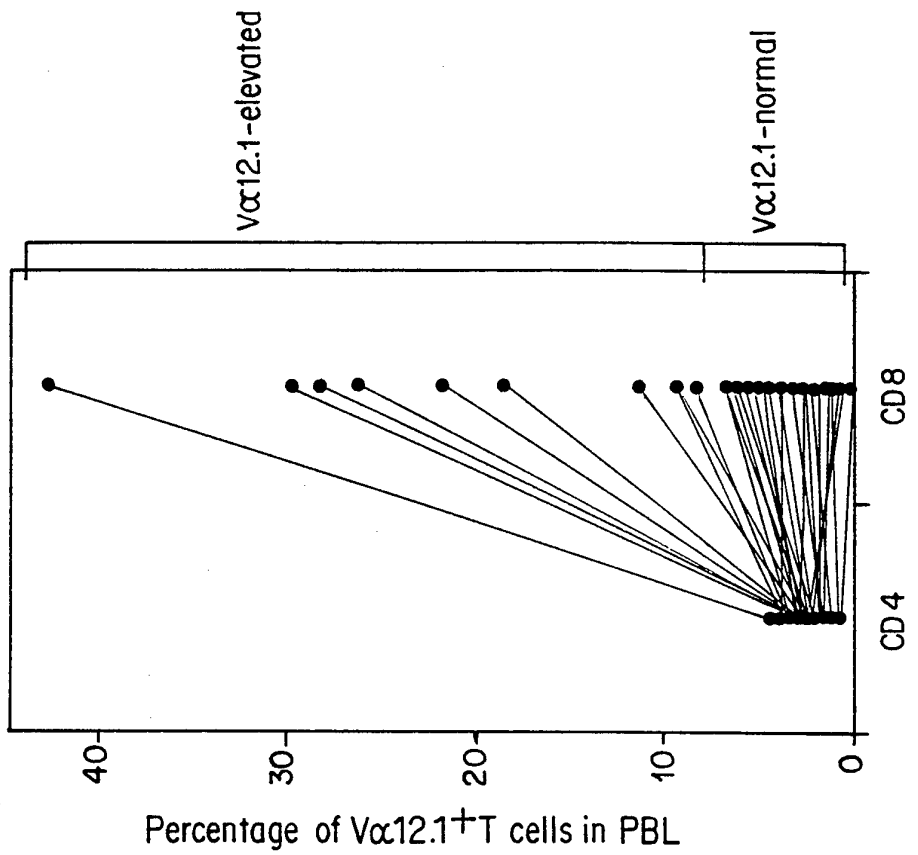

Vα12.1-elevated
Vα12.1-normal
CD8
CD4
40
30
20
10
0
Percentage of Vα12.1+T cells in PBL

FIG. 2B

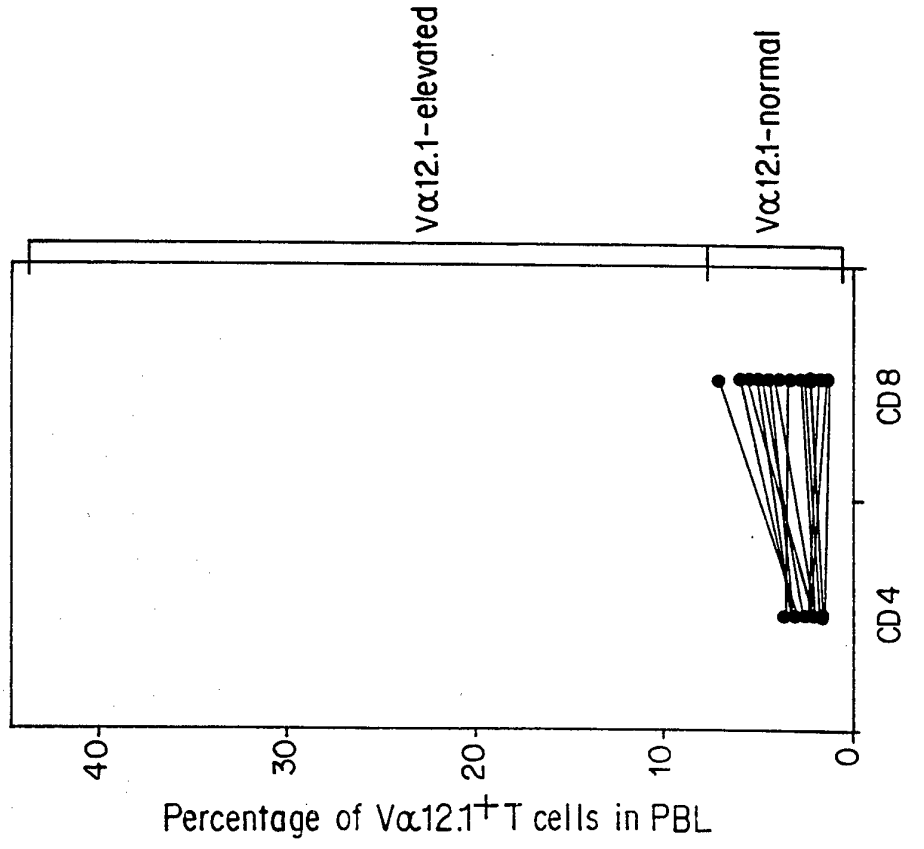

Vα12.1-elevated
Vα12.1-normal
CD8
CD4
40
30
20
10
0
Percentage of Vα12.1+T cells in PBL

FIG. 2A

DIRECT PCR

Patient 1: (Vα 12.1+/CD8+=43%)

Vα12.1: Jα Gene Sequence	# of Clones	Vα/Jα Usage
CALS DGYGQNFVFGPGTRLSVLPY	15/15	Vα12.1/JαA1

Patient 2: (Vα 12.1+/CD8+=26%)

Vα12.1: Jα Gene Sequence	# of Clones	Vα/Jα Usage
CALS DYQGGSEKLVFGKGMKLTVNPY	9/16	Vα12.1/JαA12
CALSE GAYNTNAGKSTFGDGTTLTVKPN	1/16	Vα12.1/JαA24
CAL TQTNAGKSTFGDGTTLTVKPN	1/16	Vα12.1/JαA24
CALSE VGGANNLFFGTGTRLTVIPY	1/16	Vα12.1/JαA20
CALSE PGSNNDMRFGAGTRLTVKPN	1/16	Vα12.1/JαA15
CALS GIDDKIIFGKGTRLHILPN	1/16	Vα12.1/JαP
CAL LRSGGYNKLIFGAGTRLAVHPY	1/16	Vα12.1/JαA16
CALSEA WGGSQGNLIFGKGTKLSVKPN	1/16	Vα12.1/JαA9

Patient 3: (Vα 12.1+/CD8+=28%)

Vα12.1: Jα Gene Sequence	# of Clones	Vα/Jα Usoge
CAL RGGGADGLTFGKGTHLIIQPY	16/24	Vα12.1/JαA6
CALSE PYSGGGADGLTFGKGTHLIIQPY	3/24	Vα12.1/JαA6
CALSEA PGVTDSNYQLIWGAGTKLIIKPN	1/24	Vα12.1/Jα??
CALSE PNTGRRALTFGSGTRLQVQPN	1/24	Vα12.1/Jα??
–		
–		
–		

FIG.3A

iPCR Method:

α - chains

Patient 1:

Vα12.1:	Jα Gene Sequence	# of Clones	Vα/Jα Usage
CALS	DGYGQNFVFGPGTRLSVLPY	9/9	Vα 12.1/Jα A1

Patient 3:

Vα12.1:	Jα Gene Sequence	# of Clones	Vα/Jα Usage
CAL	RGGGADGLTFGKGTHLIIQPY	21/27	Vα 12.1/Jα A6
CALSE	PYSGGGADGLTFGKGTHLIIQPY	5/27	Vα 12.1/Jα A6
–		1/27	Vα 12.1/Jα A14

β - chains

Patient 1:

Vβ:	Dβ and Jβ Gene Sequence	# of Clones	Vβ/Jβ Usage
C		18/18	Vβ 5.1/Jβ 2.7

Patient 3:

Vβ:	Dβ and Jβ Gene Sequence	# of Clones	Vβ/Jβ Usage
C		12/20	Vβ 8/Dβ/Jβ 1.2
C		1/20	Vβ 2/Dβ/Jβ 2.3
C		1/20	Vβ 3/Dβ/Jβ 2.3
C		1/20	Vβ 3/Dβ/Jβ 2.1
C		1/20	Vβ 15.1/Dβ/Jβ 2.5
C		1/20	Vβ 8/Dβ/Jβ 1.2
C		1/20	Vβ 8/Dβ/Jβ 1.2

FIG.3B

SUBSTITUTE SHEET (RULE 26)

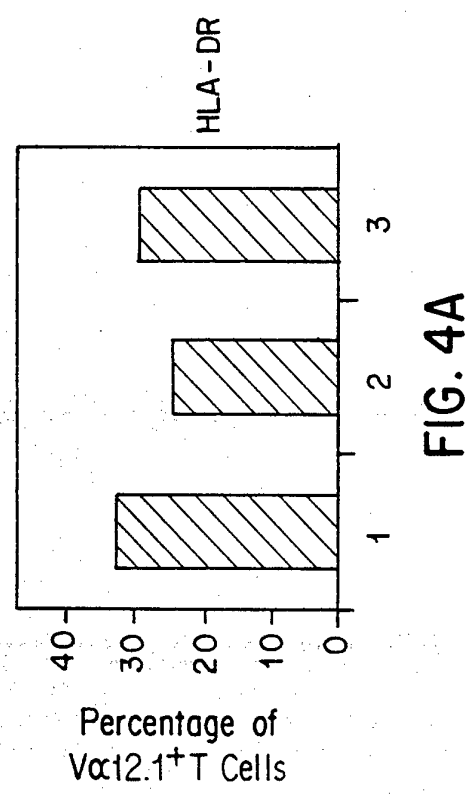

HLA-DR
1
2
3
0
10
20
30
40
Percentage of
Vα12.1+ T Cells

FIG. 4A

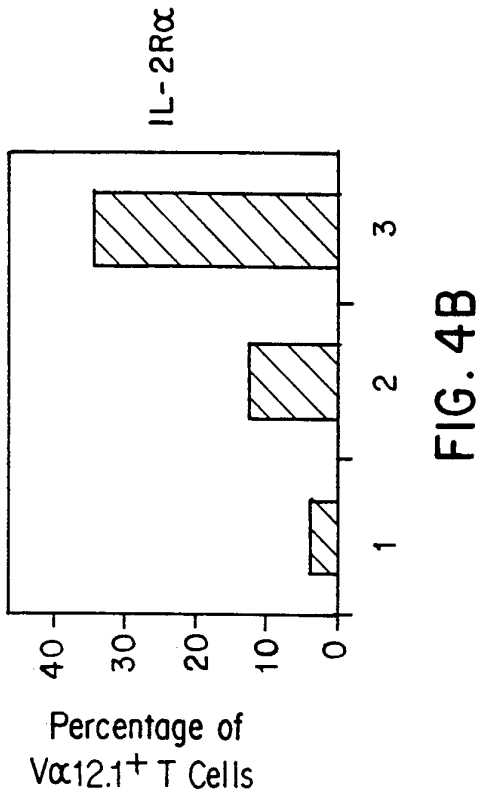

IL-2Rα
1
2
3
0
10
20
30
40
Percentage of
Vα12.1+ T Cells

FIG. 4B

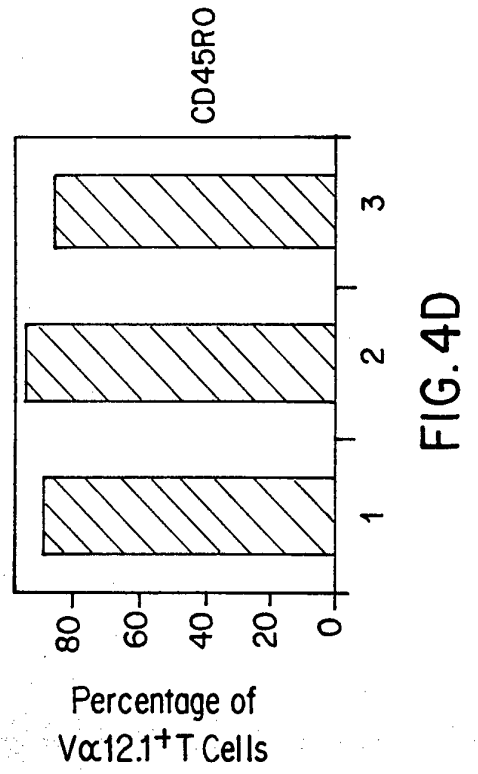


FIG. 4D

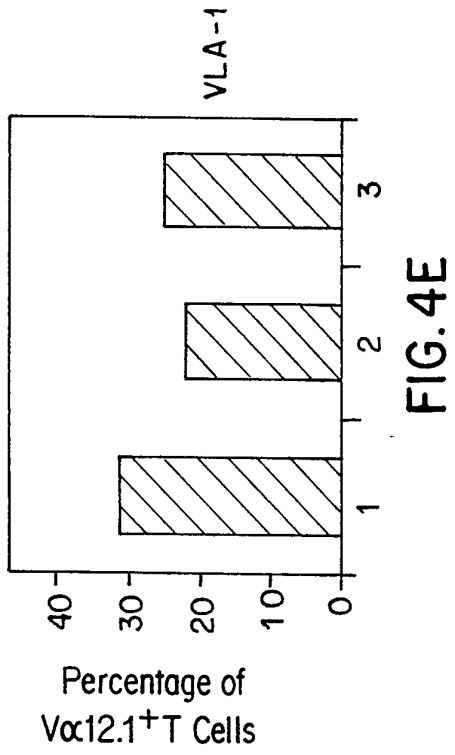


FIG. 4E

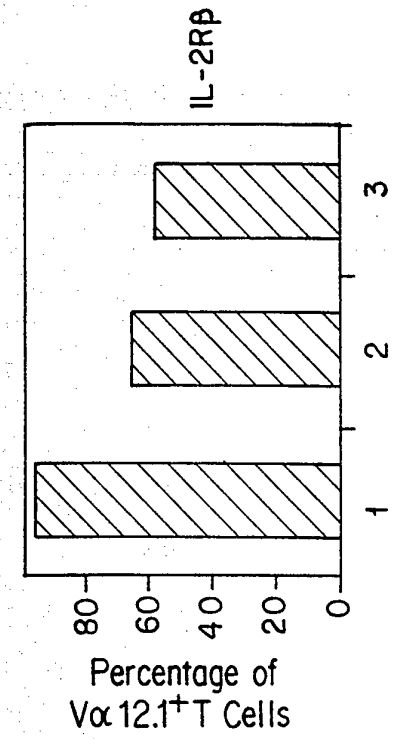


FIG. 4C

HLA Tissue Typing

RA patients:

1)		A32, B60, C3	:DR4,	
	($6D6^+/CD8^+$:43%)	AX, B44, CX	:DR7,	DQw2
2)		A2, Bw52	:DR3	DQw2
	($6D6^+/CD8^+$:22%)	A11, B48	:DR10	
3)		A1, B8	:DR3,	DQw2
	($6D6^+/CD8^+$:29%)	A2, BX,	:DR4	
		Bw6,		
4)		A2, B35, Cw4	:DR1,	DQw1
	($6D6^+/CD8^+$:27%)	A11, Bw57,Cw3	:DR2,	
		Bw4, Bw6		
5)		A23,B13,	:DR1,	DQw1
	($6D6^+/CD8^+$:26%)	A30,B14,	:DR7,	DQw2
		Bw4,Bw6		
6)		A23,B27,Cw2	:DR4, DRw53,	DQw2*
	($6D6^+/CD8^+$:22%)	A24,Bw42,	:DR5, DRw52,	
		Bw4,Bw6		

* Confirmed by DQw2-specific oligo probe
(DQw2 is linked to DR3 and DR7)

FIG.5A

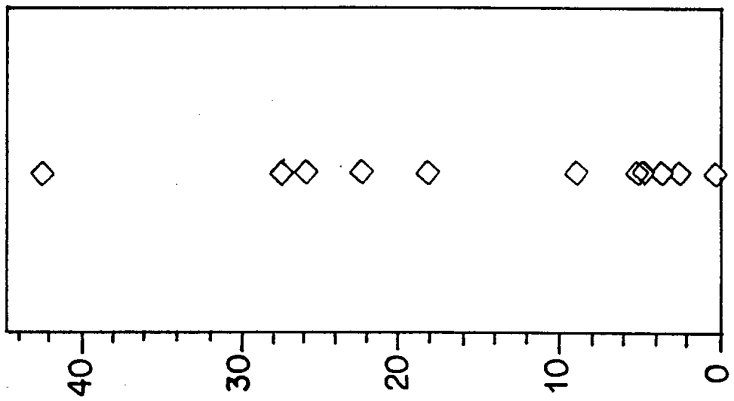

40
30
20
10
0
Percent of Vα12.1+T cells in CD8 subset

FIG. 5C

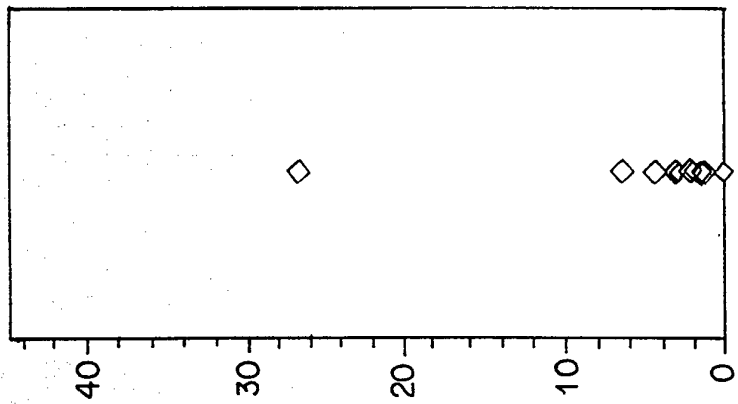

40
30
20
10
0
Percent of Vα12.1+T cells in CD8 subset

FIG. 5B

INTERNATIONAL SEARCH REPORT

Inter onal application No.
PCT/US93/08644

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :CO7K 5/04, 7/04, 15/28; A61K 37/02, 37/04, 39/395
US CL :530/300, 326, 327, 328, 387.1, 388.22; 514/2; 424/85.8
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/300, 326, 327, 328, 387.1, 388.22; 514/2; 424/85.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EMBL, GENBANK, UEMBL, DIALOG
search terms: sequence ID Nos. 1-17; autoimmunity, tcr, variable, antibody

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP, A, 0,340,109 (Steinman, et al.) 02 November 1989.	1-15
Y	Journal of Experimental Medicine, Volume 174, issued September 1991, H. DerSimonian et al., "Increased Frequency of T Cell Receptor Vα12.1 Expression on CD8+ T Cells: Evidence that Vα Participates in Shaping the Peripheral T Cell Repertoire", pages 639-648, see especially page 640, lines 3-15 and the first three paragraphs of the Discussion at pages 645-646.	1-15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 08 December 1993	Date of mailing of the international search report 16 DEC 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer GEORGE C. ELLIOTT Telephone No. (703) 308-0196

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