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(57) Abstract

This invention relates to a method for diagnosis of rheumatoid arthritis in an individual. This method is based on the discovery that the presence of a viral peptide or protein is associated with the occurrence of rheumatoid arthritis in an individual. The invention also relates to the treatment of rheumatoid arthritis by administration of an anti-viral agent.

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DIAGNOSTICS AND THERAPY FOR RHEUMATOID ARTHRITIS

1. INTRODUCTION

This invention relates to a method for diagnosis of rheumatoid arthritis in an individual. This method is based on the discovery that the presence of a viral peptide or protein is associated with the occurrence of rheumatoid arthritis in an individual.

The invention also relates to the treatment of rheumatoid arthritis by administration of an anti-viral agent.

2. BACKGROUND OF THE INVENTION

2.1. RHEUMATOID ARTHRITIS

2.1.1 RHEUMATOID ARTHRITIS PATHOLOGY

Rheumatoid arthritis (RA) is a chronic systemic disorder of unknown etiology, whose histopathology is characterized by proliferation of synovial cells, destruction of articular cartilage and bone, and mononuclear cell infiltration. Most of its serious and debilitating sequelae are derived from erosion and destruction of articular connective tissues.

Animals of the autoimmune MRL/1 mouse strain (Murphy, E.D. and Roths, J.B., 1978, in Genetic Control of Autoimmune Disease, Rose, N.R., et al., eds., Elsevier/North-Holland, New York, pp. 207-219) develop a spontaneous rheumatoid arthritis-like disease (Hang, L., et al., 1982, J. Exp. Med. 155:1690-1701). The pathologic changes that occur in the joints of these mice can be divided into three stages (O'Sullivan, F.X., et al., 1985, Arthr. Rheum. 28:529-536). The first stage develops between the ages of 7 and 13 weeks and consists of synovial

cell proliferation in the joint recesses. The second stage is characterized by continued proliferation of synovial cells which take on an appearance similar to transformed mesenchymal cells. The earliest destructive changes occur in the second stage and include marginal erosions, followed soon after by progressive destruction of articular and meniscal cartilage. The final stage is characterized by a diminution of synovial cell proliferation, extensive cartilage destruction, formation of scar tissue and fibrocartilage, and a moderate infiltration of the synovial stroma by mononuclear and polymorphonuclear inflammatory cells.

Throughout the progression of MRL/1 disease there is a striking dissociation between inflammatory cell infiltration or exudation and tissue destruction. The erosions of articular cartilage and subchondral bone are initially associated with proliferation, attachment and invasion by transformed-appearing synovial lining cells resembling the primarily noninflammatory joint destruction described in human RA (Fassbender, H.-G., 1983, Collagen Rel. Res. 3:141-155). Typical erosions of articular structures in MRL arthritis and human RA occur predominantly in areas contiguous with the proliferating synovial cells, suggesting release of enzymes from the proliferating cells capable of degrading components of the extracellular matrix, in particular the collagens of cartilage and bone (Krane, S.M. and Amento, E.P., 1983, J. Rheumat. 10 (Supp. II):7-12). In this regard the potential major role of collagenase and its regulation have been explored extensively (Harris, E.D., et al., 1984, Collagen Rel. Res. 4:493-512). A substance responsible for increased collagenase and Prostaglandin E_2 production by synovial cells has been identified and termed mononuclear cell factor, or MCF (Dayer, J.M., et al., 198I, FEBS Lett. 124:253-256).

Although a number of cellular interactions among cells derived from rheumatoid synovial membranes has been described, the exact mechanism of primary joint destruction remains unknown. Why synovial cells proliferate in the absence of inflammatory cell infiltrates (O'Sullivan, F.X., et al., 1985, Arthr. Rheum. 28:529-536, Fassbender, H.-G., 1983, Collagen Rel., Res. 3:141-155) and why these cells begin to attach to and subsequently degrade the connective tissue matrix of cartilage and bone remained key questions in the art.

The mechanism of cellular attachment is of particular interest, especially since erosions of cartilage and bone occur in the MRL/1 mice in the early inflammatory stage of disease only in areas where the proliferating synovial lining cells appear in close contact to cartilaginous and bony matrices (O'Sullivan, F.X., et al., 1985, Arthr. Rheum. 28:529-536). The same is true for the primary lesions in human rheumatoid arthritis (Fassbender, H.G., 1983, Collagen Rel. Res. 3:141-155). These early stages need to be clearly separated from observations made in later stages that are characterized by the presence of an inflammatory vascularized pannus and by production of soluble mediators and proteolytic enzymes (Ziff, M., 1983, J. Rheumat. 10 (Supp. 11):13-25; Dingle, J.T., 1983, J. Rheumat. 10 (Supp. 11):38-42).

Electron microscopy of normal synovial lining in human (Wynne-Roberts, C.R. and Anderson, C., 1978, Arthr. Rheum. 7:279-286; Ghadially, F.N., 1980, Ultrastruct. Path. 1:249-264), rat (Graabaek, P.M., 1984, Lab. Invest. 50:690-702), and mouse (Linck, G. and Porte, A., 1978, Cell Tissue Res. 187:251-261; Linck, G. and Porte, A., 1978, Cell Tissue Res. 185:251-261) joints has revealed type A cells, which are macrophage-like phagocytic cells, and type B cells, which are more fibroblastic. It has been suggested that proliferating synovial cells in the MRL/1

mouse (O'Sullivan, F.X., et al., 1985, Arthr. Rheum. 28:529-536) as well as in human rheumatoid arthritis (Fassbender, H.G., 1983, Collagen Rel. Res. 3:141-155) have undergone transformation.

Viruses such as Epstein-Barr virus, hepatitis B virus, rubella virus, reovirus, and parvovirus (Brown, K.A., 1984, Nature 309:582) have been implicated in the etiology of rheumatoid arthritis (see Sokoloff, L., 1984, Intl. Rev. Exp. Pathol. 26:117-119; Smith, C.A., 1979, J. Rheumatol. 6(2):113-116; April 7, 1984, The Lancet, pp. 772-774; Bacon, P.A. and Tunn, E.J., 1986, Quarterly J. Med., New Series 61 (234):897-899; Snyderman, R., et al., 1987, Arthritis and Rheumatism 30(10):1191-1194). Chronic arthritis in goats appears to be caused by caprine arthritis-encephalitis virus (CAEV), a lentivirus member of the retrovirus group (Crawford, T.B., et al., 1980, Science 207:997; Banks, K.L., et al., 1987, Arthritis and Rheumatism 30(9):1046-1053; Brassfield, A.L., et al., 1982, Arthritis and Rheumatism 25(8):930-936).

2.1.2 TREATMENTS FOR RHEUMATOID ARTHRITIS

Although the etiology of rheumatoid arthritis (also equivalently referred to as "RA") was heretofor unknown, various treatments of the disease's pathologic characteristics have been undertaken. Salicylates, which include aspirin, are used for their anti-inflammatory and analgesic properties. Ibuprofen and similar compounds are salicylate alternatives. Indomethacine is another anti-inflammatory and analgesic drug which is useful for those patients who are not good candidates for treatment with the salicylates. Gold compounds are used in addition to the prior noted compounds against active joint inflammation, but the compounds are not usually effective where the RA condition is advanced. In addition, toxic side reactions have resulted from the use of gold compounds.

D-Penicillamine, which is used as an alternative to the gold compounds, is also capable of serious toxic side reactions. Mild to moderate active RA symptoms are treatable with hydroxychloroquine, although irreversible retinal damage can occur with its use. The use of corticosteroids provides short term anti-inflammation effects, but the progression of joint damage is not affected. (Berkow, R., et al., 1982,The Merck Manual, 779-786.)

2.2. RETROVIRUSES

RNA tumor viruses constitute the Oncovirinae subfamily of the Retroviridae family. The Retroviridae, or retroviruses, are enveloped RNA viruses (for a review, see Hayward, W.S. and Neel, B.G., 1981, Curr. Top. Microbiol. Immunol. 91:217-276) which utilize a DNA intermediate in their replication cycle. The virus particle consists of a ribonucleoprotein core enclosed by an outer membrane envelope derived from the host cell plasma membrane. Viral envelope glycoproteins protrude from the outer envelope. The viral genome consists of a single-stranded RNA molecule.

All retrovirus families and subfamilies replicate via a DNA intermediate, as a result of the action of the enzyme RNA-dependent DNA polymerase, or reverse transcriptase. The replication cycle begins with adsorption of the virus to the cell surface, followed by penetration of the cell membrane, uncoating of the virus particle, and the release of viral RNA. The viral RNA then serves as the template for the synthesis of DNA by reverse transcriptase. Some of the linear DNA duplexes are transported to the nucleus where they are converted to covalently closed, circular DNA molecules (proviral DNA), which are then integrated into the host cell chromosomal DNA. The integrated proviral DNA replicates with the host

genome, producing genomic RNA for incorporation into virus particles, and serving as the template for the synthesis of viral messenger RNAs which are processed and translated to produce the viral proteins. Assembly of the virus particle occurs in the cytoplasm at the cell membrane. The immature particles bud through the membrane into the extracellular environment where maturation occurs. The released particles are then ready to begin a new round of infection.

The avian RNA tumor viruses can be divided into two groups based on their pathogenic properties. The rapidly transforming viruses are capable of producing neoplasia in vivo within 2-4 weeks and will morphologically transform cells in vitro (Graf, T. and Beug, H., 1978, Biochem. Biophys. Acta Rev. Cancer 516:269-299). The slowly transforming retroviruses induce neoplasia after 4-12 months, and do not transform tissue culture cells (Purchase, H.G., et al., 1977, Infect. Immun. 15:423-428). The differences in pathogenic properties have a genetic basis: the acutely transforming retroviruses contain transforming genes that are not present in the genomes of the slowly oncogenic viruses.

Avian leukosis virus, or ALV, is the prototype of the slowly transforming retrovirus. Its genome is composed of the gag, pol, and env genes in a 5′ to 3′ linear array. The gag gene encodes the structural proteins of the virus core, or group-specific antigens, pol encodes the reverse transcriptase, and env encodes the envelope glycoproteins.

Studies have demonstrated that each of the known retroviral oncogenes, or v-onc, has a normal cellular counterpart or c-onc. The transforming proteins encoded by the oncogenes encompass a variety of cellular locations and functions (for review, see Hunter, T., 1984, Sci. American 251:70-79). They can be divided into groups on the basis of sequence and functional similarities. For example, the ras proteins have a GTP-binding property, while both myc

and myb (Klempnauer, K.-H. and Sippel, A.E., 1986, Mol. Cell. Biol. 6:62-69) encode nuclear proteins with DNA-binding activity. Several transforming proteins have been shown to be related to growth factors or their receptors.

The major ras-induced protein in NIH3T3 (mouse fibroblast) cells is the cysteine protease cathepsin L (Joseph, L., et al., Nucl. Acids Res. 15(7):3186). A positive correlation has been found between the extent of ras expression, the metastatic potential, and the amount of secreted cathepsin L in H-ras-transformed murine fibroblasts (Denhardt, D.T., et al., 1987, Oncogene 2:55-59). The major excreted protein from a Kirsten virus (carrying the ras oncogene)-transformed mouse fibroblast line appears to be a catalytically active precursor form of cathepsin L (Mason, R.W., et al., 1987, Biochem. J. 248:449-454).

Human T Cell Leukemia Virus (HTLV) are a family of related human retroviruses that exhibit a tropism for human T cells. HTLV-I and HTLV-II are associated with certain leukemias and lymphomas. HTLV-I is causatively linked to adult T cell leukemia (Gallo, R.C. and Wong-Staal, F., 1982, Blood 60:545; Gallo, R.C., 1984, in Cancer Surveys, Vol. 3, Franks, L.M., et al., eds., Oxford Univ. Press, Oxford, pp. 113-159). HTLV-II was first identified in a patient with a T cell variant of hairy cell leukemia (Kalyanaraman, V.S., et al., 1982, Science 218:571). Both viruses have the capacity to transform infected T cells in vitro, in addition to causing other cellular changes (see Arya, S.K., et al., 1984, Science 225:927-930, and references cited therein).

An HTLV-I-like retrovirus has been isolated from T cell lines derived from patients with tropical spastic paraparesis, a progressive myelopathy (Jacobson, S., et al., 1988, Nature 331:540-543). Data has suggested that multiple sclerosis is associated with the presence of a

novel retrovirus related to HTLV-I (Koprowski, H., et al., 1985, Nature 318:154-160), but further studies have failed to demonstrate any involvement of an HTLV-I-like virus (Hauser, S.L., et al., 1986, Nature 322:176-177; Karpas, A., et al., 1986, Nature 322:177-178).

The causative agent of AIDS is a retrovirus, now termed human immunodeficiency virus type 1 (HIV-1), and formerly termed HTLV III (Gallo, R.C., et al., 1984, Science 224:500; Popovic, M., et al., 1984, Science 224:497), LAV (Barre-Sinoussi, F., et al., 1983, Science 220:868; Feorino, P.M., et al., 1984, Science 225:69), and ARV (Levy, J.A., et al., 1984, Science 225:840) by the three groups which independently isolated viruses which are probably of the same retrovirus subgroup (Levy, J.A., et al., Science 225:840).

The F (3' orf gene) of HIV (HTLV-III) has been shown to encode a protein, with sequence homologies to oncogene products, that exhibits the GTPase, autophosphorylation, and GTP-binding activities associated with the ras protein (Guy, B., et al., 1987, Nature 330:266-269).

Although Pelton, B.K., et al., 1988, Annals Rheu. Diseases 47:206-209 found no evidence of viral infection in patients with a RA or systemic lupus erythematosis (SLE) using reverse transcriptase production and hybridization with HIV cDNA probes, lentiviruses have been studied in connection with those disorders since lentivirus induced infections and diseases exhibit many immunpathological characteristics similar to those associated with RA and SLE. Evidence suggests that lentiviral infection is horizontally transmitted (Yanin, A., et al., 1985, Virology 145:340-345), since no endogenous lentivirus or other lentiviral genomic material has been demonstrated (1979, Intervirology 12:234-239). Visna virus, one of the

lentivirus species, has been shown to contain a major internal structural polypeptide, p30, which has group-specific antigenic determinants common to other lentiviruses which are not found in other retroviruses such as oncoviruses or spumaviruses (1979 Intervirology 12:234-239). Nucleotide sequence analysis of various viral genomes (Chiu, I.-M., et al., 1985, Nature 317:366-368) demonstated that HTLV-III was most closely related to the lentivirus CAEV, whereas the genomes of HTLV-I and HTLV-II were most closely related to the oncovirus subfamily.

No specific viral or viral-like components have been confirmed as etiologic factors in RA. Consequently, methods for diagnosing RA have centered on examinations of secondary pathologic features. In addition, treatments of RA have been directed at alleviating those secondary pathologic features.

Clearly, what is needed is a better method to assay whether a RA condition exists in an individual, premised on the etiology of the disease. In addition, a clearer understanding of RA's etiology can provide an improved treatment protocol.

3. SUMMARY OF THE INVENTION

This invention relates to a method for diagnosis of rheumatoid arthritis in an individual. This method is based on the discovery that the presence of a viral peptide or protein is associated with the occurrence of rheumatoid arthritis in an individual. Generally stated, this invention comprises an assay to diagnose RA in a patient suspected of having RA.

More specifically, the invention relates to the treatment of rheumatoid arthritis with an anti-viral agent. Such a treatment is directed at the biological particles now known to be associated with the disease's etiology.

4. BRIEF DESCRIPTION OF THE FIGURES

This invention may be more readily understood by reference to the following detailed description of the invention and figures, in which:

FIGURE 1 shows fresh frozen section of synovial membrane (233/31) of patient with RA stained with monoclonal antibody to HTLV-I p19 and secondary FITC-labeled goat - antimouse IgG. Less than 5% of synovial cells show specific intracellular and/or membraneous staining. (x 400)

FIGURE 2 shows paraffin section of formalin-fixed RA synovial tissue (R 1892) stained with monoclonal antibody to HTLV-I p19 labeled by the immunogold silver enhancement technique. Specific staining of synovial cells adjacent to the site of bone erosion is shown. (x 250)

FIGURE 3 is an electronmicrograph which shows a typical retroviral structure observed in a sample from a patient afflicted with RA. The serum from this RA patient was negative for antibodies against HTLV-I and HIV-1. (x 285,000)

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods for diagnosing rheumatoid arthritis in an individual. The invention also provides methods for treating rheumatoid arthritis by administration of an anti-viral agent.

Generally stated, it has been discovered that an individual with rheumatoid arthritis contains retroviral protein sequences such as HTLV-related protein sequences which are associated with the proliferating and transformed-appearing synovial cells and immunological response and inflammation of the joint. These protein sequences comprise an epitope that can be recognized by antibody directed against a protein determinant of HTLV. The detection of HTLV-related peptides or proteins associated with rheumatoid arthritis provides means 1) for diagnosis of rheumatoid arthritis in an individual, and 2) for treating rheumatoid arthritis by administration of anti-viral agents.

More specifically, the preferred embodiment of this invention pertains to a method of diagnosing rheumatoid arthritis, by detection in an individual of a peptide or protein comprising an epitope related to that of an retroviral protein such as an HTLV protein. Such a method comprises:

a) obtaining a sample from the individual;

b) contacting the sample with an antibody directed against an epitope of a retroviral protein under reaction conditions that permit the antibody to bind the epitope;

c) removing unbound antibody molecules from the sample; and

d) examining the sample for the presence of bound antibody,

wherein the binding of the antibody indicates the presence of rheumatoid arthritis in the individual.

In a preferred embodiment, a monoclonal antibody directed against an envelope glycoprotein of HTLV is used.

The monoclonal antibody can be labeled. Alternatively, the method may further comprise addition of a labeled binding partner of the antibody.

The monoclonal antibody for use in the present invention is preferably selected from the group consisting of anti-HTLV I p19, p24, and anti-HTLV III p24 and gp120 as well as anti-HTLV reverse transcriptase. However, any monoclonal antibody having affinity for HTLV may be utilized in carrying out this method. The preferred antibodies are commercially available from Dupont (Billerica, MA) and are identified as anti-HTLV I p19, anti-HTLV I p24, anti-HTLV III p15/17, anti-HTLV III p24 and anti-HTLV III gp120 as well as anti-HTLV reverse transcriptase.

The peptide or protein comprising an epitope related to that of the HTLV protein can be: 1) uncomplexed; 2) complexed with biological material from said individual; 3) associated with a virus; and 4) a protein of HTLV I, HTLV III, a new HTLV-III-related protein or a new HTLV-I-related retrovirus. More specifically, the antibody can be directed against an HTLV protein that is selected from the group consisting of HTLV I p19, HTLV I p24, HTLV III p15/17, HTLV III p24, and HTLV III gp120 as well as anti-HTLV reverse transcriptase.

Regarding the peptides or proteins detected according to this embodiment of the present invention, the uncomplexed protein is a protein which is neither covalently or ionically associated with any other biological material. In other instances, protein is complexed with other biological materials via covalent or ionic bonds. In addition, the proteins can be associated with viral replication proteins or proteins produced from viral genomic material inserted into the host genome.

Samples which can be assayed include but are not limited to synovium cells, tissue and fluid, and other joint components such as meniscal and articular cartilage and subchondral bone which are in proximity with the synovium tissue and fluid.

In this invention, the preferred sample for assay is synovial material, wherein the synovial material is selected from the group consisting of the synovial cells and fluid. However, other sources of biological samples may be utilized from an affected individual (i.e., mononuclear peripheral blood cells).

Assaying for the labeled complex is preferably effected by: 1) staining methods such as a) immunofluorescence (Figure 1), b) an immuno-peroxidase technique and c) immunogold staining with silver enhancement (Figure 2), and 2) an ultrastructural analysis by electron microscopy (as shown in Figure 3). In addition, immunocytology, immunoblotting, and ELISA (enzyme-linked immunosorbent assay) methods are also used.

In another embodiment of the invention, rheumatoid arthritis can be diagnosed in a patient by an in situ hybridization assay, using as probe a labeled nucleic acid sequence encoding an retroviral protein such as an HTLV protein. Such nucleic acid probes include but are not limited to DNA, cDNA, or RNA sequences which can encode at least a portion of an envelope glycoprotein, core protein, polymerase, reverse transcriptase or other retrovirus-specific-protein.

More specifically, this method is disclosed of diagnosing rheumatoid arthritis in a patient comprising:

(a) isolating a tissue sample from the patient;

(b) contacting the tissue sample with a nucleic acid probe specific for a retroviral gene, under conditions that allow hybridization of the probe to nucleic acids in the sample;

(c) removing unbound probe; and

(d) detecting the hybridization of the probe,

in which hybridization of the probe is indicative of rheumatoid arthritis in the patient. In particular, the retroviral gene is an HTLV gene.

Alternatively, the method of diagnosis may comprise isolation of a sample of nucleic acid from the patient followed by hybridization to a nucleic acid probe specific for a retroviral gene. In particular, such a method may comprise a Northern or Southern hybridization procedure or a cyto dot blot hybridization technique.

Another embodiment of this invention is a method for treating rheumatoid arthritis in a patient by administering an anti-viral agent to the patient. The anti-viral agent is preferably selected from the group consisting of acyclovir, phosphonoformate, ribavirin, "anti-sense" oligodeoxy nucleotide, protease inhibitor, 2',3'-dideoxynucleoside, methylphosphonate oligonucleotide and interferon. In addition, said 2',3'-dideoxynucleoside is selected from the group consisting of 3'-azido-2',3'-dideoxythymidine and 2',3'-dideoxycytidine.

6. EXAMPLES

6.1 IMMUNOHISTOLOGICAL APPLICATION OF MONOCLONAL ANTIBODIES AGAINST RETROVIRAL PROTEINS

6.1.1 IMMUNOFLUORESCENT STAINING OF BIOPSIED TISSUE SECTIONS

6.1.1.1 IMMUNOFLUORESCENCE

Sections of tissues 4-6 um thick are prepared from frozen, unfixed biopsy samples by cryostat sectioning. The air-dried sections are incubated with an appropriate monoclonal antibody. For controls, sections are incubated with immunoglobulin (Ig) from pre-immune serum. After 30 minutes of incubation in a humidified chamber at room temperature, the sections are rinsed three times with phosphate-buffered saline (PBS, pH 7.4) and, in a second step, layered with a 1:30 dilution of fluorescein-isothiocyanate (FITC) conjugated rabbit anti-mouse Ig for 30 minutes. Finally, the slides are washed exhaustively to remove nonspecifically associated reagents and are sealed with a solution of 90% glycerol/10% PBS under a coverslip. The localization of staining is observed and photographed using a Leitz-fluorescence microscope equipped with a K2 filter system for FITC.

6.1.1.2 IMMUNOPEROXIDASE TECHNIQUE

Paraffin-sections are deparaffinized in xylene and alcohol. After trypsin digestion (0.1 mg/ml, Tris-HCl, 60 min), the sections are treated with 0.3% hydrogen peroxide to inactivate endogenous peroxidase activity and rinsed in 0.05% saponin (2 x 15 min each). Subsequently, sections are pretreated with normal serum diluted 1:5 in 0.2% BSA/PBS, washed and incubated with the primary antibody at 4°C overnight. After washing

the ABC technique is utilized as described (HSU et al., 1981, J. Histochem. Cytochem 29:557-580), utilizing a VECTASTAIN-ABC Kit (Vector Lab., Inc., Burlingame, CA).

6.1.1.3 IMMUNOGOLD STAINING WITH SILVER ENHANCEMENT (IGSS)

Paraffin sections are deparaffinized and hydrated as mentioned above. Sections are incubated for 30 min with 5% heat-inactivated blocking serum (goat) and subsequently incubated overnight at 4°C with monoclonal antibody. Thoroughly washed slides are covered with gold-conjugated antibody (AuroProbe LM goat anti-mouse IgG (Janssen, Life Sci. Products, Olen, Belgium), diluted 1:40 with PBS, pH 7.3, containing 0.1% BSA, and are incubated for 2 hours at room temperature.

After washing with PBS and distilled water the sections are covered with freshly prepared silver enhancement reagent (IntenSE II (Janssen, Life Sci. Products, Olen, Belgium) and the reaction is monitored under a light microscope.

After 5 to 15 min the reaction is stopped by washing with distilled water and the sections are counterstained with hematoxylineosin, cleaned and mounted.

6.1.2 IMMUNOELECTRONMICROSCOPY

When immunoelectronmicroscopy is performed on synovial biopsies, the specimens are cut into slices 0.2 to 0.5 mm thick with 2 razor blades or with a cryostat. They are fixed in 1% glutaraldehyde in 0.16 M cacodylate buffer, pH 7.4, at 4°C. for 20 minutes [R. Fleischmajer et al., J. Invest. Dermat. 75:189-191 (1980)], washed several times eith 0.15 M Tris-HCl buffer, pH 7.5 to remove the excess glutaraldehyde and stored in that buffer at 4°C. Incubations with monoclonal antibodies

are carried out for 24 hours at 4°C. with rabbit anti-mouse Ig antibodies tagged with ferritin, diluted 1:5 in PBS, pH 7.2. The specimens are rinsed with 0.1 M Tris-HCl buffer, pH 7.5 for 24 hr at 4°C, washed 3 times with 0.16 M cacodylate buffer, pH 7.5, and fixed in 3% glutaraldehyde in 0.16 M cacodylate buffer, pH 7.4, for 2 hr. Control specimens are treated only with rabbit anti-mouse Ig ferritin-tagged antibodies. The specimens are postfixed in osmium tetroxide, passed through graded alcohols and embedded in Araldite. The blocks are sectioned in a LKB ultratome-4 microtome and the specimens, about 500-600 Å (angstrom) thick, are either left unstained or are stained with a saturated solution of uranyl acetate. The grids are examined with a Hitachi HU-12A or with a Phillips 300 electron microscope.

6.1.3 IMMUNOCYTOLOGY

Other cells, for instance isolated synovial cells or mononuclear peripheral blood cells, are fixed in phosphate buffered 4% paraformaldehyde at 4°C. for 2 hours with one change of solution [Gay et al., Collagen Rel. Res. 1:370-377 (1981)]. Cells are then washed for 36 hours in PBS with 4% sucrose at 4°C with multiple changes.

The last wash is performed in PBS with 4% sucrose and 5% glycerol for 1 hour. Cell pellets are then placed in OCT freezing medium with a cork or plastic backing to hold them and quickly frozen by immersing them in a jar of methylbutane (isopentane) placed in a small chamber of liquid nitrogen. The frozen cells are then wrapped in aluminum foil and stored in a closed container at -20°C. Frozen sections 8 um thick are cut and placed in albumin coated slides and air-dried for at least 5 minutes. Slides are then

placed in a solution of ice-cold $NaBH_4$ (10 mg/100 ml) in PBS for 1 hour with one change. Following this procedure, the slides are washed at 4°C in PBS, 3 changes for 30 minutes each.

Cell sections are reacted with the appropriate monoclonal antibody in a moist chamber overnight at 4°C or at room temperature for 2 hours. Slides are washed thoroughly with PBS and then incubated an additional 2 hours with secondary antibody (goat or rabit anti-mouse Ig). This is followed by washing with cold PBS and a third antibody treatment with Fab-peroxidase-anti-peroxidase (Fab-PAP) for 3 hours. The Fab-PAP solution is removed by washing with PBS and the tissue sections are incubated in 150 ml of 0.1 M Tris, pH 7.6, containing 40 mg of 3,3-diaminobenzidine tetrahydrochloride and 15 ul of 5% H_2O_2 for 15-18 minutes. Slides are then washed with cold PBS and stained with 1% osmium tetroxide for 1 hour at room temperature. The stained slides are again rinsed with cold PBS, dehydrated in acetone, embedded in Maraglas® (70%) and ultra-thin sections are made for examination using an electron microscope.

6.2 IMMUNOBLOTTING

Protein immunoblot analysis is performed to confirm antibody reactivity seen in immunofluorescent studies. Primary synovial cell cultures are grown-up in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum. Adherent cells from duplicate dishes are treated as follows: (1) One dish is washed with PBS and cells are released by treatment with 0.25% trypsin in PBS. Harvested cells are counted to determine total cell number per dish. (2) A duplicate dish of cells, which has not been treated with trypsin, is washed with PBS and cells are harvested directly into

SDS-polyacrylamide gel electrophoresis sample buffer (containing 0.1% SDS/4 M urea) at a final concentration of 10^6 cells per ml sample buffer. Solubilized cell proteins (an equivalent of 10^5 cells) are separated electrophoretically in a polyacrylamide gel and transferred to nitrocellulose. To limit nonspecific antibody binding, each blot is preincubated in a 1% bovine serum albumin (BSA) solution. Monoclonal antibodies are diluted in 1% BSA solution and incubated with blots to allow antibody binding. Bound antibody is detected by incubation with a peroxidase-conjugated goat-anti-mouse antibody (also diluted in 1% BSA solution) and visualized with 4-chloro-1-naphthol. A duplicate blot is stained with an amido black solution to visualize total transferred proteins as well as molecular weight markers.

6.3 ELISA FOR THE DETECTION OF CIRCULATING ANTIBODIES

6.3.1 ELISA

Ninety-six-well microtiter plates (Dynatech Immulon) are coated overnight with fresh dilutions of highly purified stock solutions of retroviral protein in 0.02 M Na_2CO_3 (pH 9-10). Wells are then washed 3 times with phosphate-buffered saline containing 0.05% Tween 20 and 1% Merthiolate (PBS-Tween) for removal of unbound protein. The plates are then blocked with 0.5% bovine serum albumin in PBS (PBS-BSA) and subsequently washed with PBS-Tween.

6.3.2 PATIENT SERA

Sera from patients with rheumatoid arthritis are obtained by bleeding. A single lot of pooled normal human serum is used for negative controls.

Patient sera are diluted 1:1, 1:10, 1:100 in PBS and 50 μl of each diluted serum is added to triplicate wells and incubated at 37°C for 1 hr. The wells are then washed three times with PBS-Tween, and 50 ul of horseradish peroxidase (HRP) conjugated goat anti-human immunoglobulin (New England Nuclear Screening System NE1-602) diluted 1:500 is added to each well and incubated for 1 hr at 37°C. After five washes to remove unbound peroxidase-conjugated antibody, 50 ul of freshly prepared substrate (o-phenylenediamine) is added to the wells and incubated for 15 min at 37°C. The reaction is then quenched with 4.5 M H_2SO_4, and absorbance of each sample is read on an automated ELISA reader (Titer Tek Multiscan) at 492 nm.

Alternatively, ^{125}I-labelled sheep antihuman immunoglobulin (specific activity, 5-20 μC/μg obtained from Amersham, Arlington Heights, JL.) is diluted and aliquoted so as to give 50,000 counts/well and then the resultant wells are incubated for an additional hour at 37°C. Unbound radioactivity is removed by extensive washing with PBS plus 0.05%. Tween and the radioactivity in each well is then counted in a Beckman 5500 gamma counter.

6.4 RESULTS REGARDING DIAGNOSING FOR RHEUMATOID ARTHRITIS

Diagnosing for a retroviral, and particularly an HTLV connected etiology for rheumatoid arthritis has proven to be successful. In one study, the expression of retroviral products in about 90% of fresh synovial tissues of patients with early destructive rheumatoid arthritis was determined by immunofluorescence utilizing monoclonal antibodies to HTLV I p19 and p24. In another study, expression of retroviral products was observed in about 70% of fresh, unfixed synovial tissue samples using immunofloresence utilizing monoclonal antibodies to HTLV-I

p19 and p24 as well as HIV-1 p15/17. No expression of retroviral products was noted in patients with non-rheumatoid arthritis diseases. When the immunogold staining with silver enhancement assay was used with formalin fixed and paraffin embedded rheumatoid arthritis synovial tissues 40% of the patients' tissues stained positive, whereas no specific staining was detected in all the non-rheumatoid arthritis synovial tissues tested.

No antibodies against HTLV-I or HIV-1 were observed in sera from RA patients using ELISA or Western immunoblotting techniques. However, in one RA case, despite negative results in sera using ELISA or Western blots, examination of synovial tissue using the immunogold technique for immunoelectron microscopy, showed the presence of a typical retroviral structure (FIGURE 3). The structure having a diameter of about 65 nm comprised an electron-dense core surrounded by a double membrane that was labeled with antibody specific for HIV-1 p15/17.

Reverse transcriptase could be detected in about 70% of synovial tissues from RA patients utilizing a monoclonal antibody specific for HIV-1 reverse transcriptase (Cellular Products, Inc., Buffalo, NY).

6.5 TREATMENT OF RA

The treatment of rheumatoid arthritis in this invention utilizes an anti-viral agent, such as 3'-azido-2',3-dideoxythridine (Zidovudine). This agent has been shown to be active against many human and animal retroviruses *in vitro*, such as HTLV-I and HTLV-III, has been particularly examined regarding its cytopathic effect on reverse transcriptase activity and on retroviral antigens such as p24 core antigen (gag protein). The p24 core antigen expression was not detectable when 0.13 μg/mL of zidovudine was used in connection with a strain of HIV (HTLV-IIIB).

Zidovudine is administered orally. The drug has also been given by intravenous infusion, although no parenteral dosage form of the drug is currently commercially available in the U.S. The initial oral dosage of zidovudine currently recommended is 200 mg every 4 hours 6 times daily; however, this recommendation is based on limited experience, and the optimum dosage and schedule of administration have not been fully established. This dosage corresponds to 2.9 mg/kg every 4 hours in a 70-kg patient. In clinical trials, zidovudine usually was administered orally in dosages of 250 mg (2.3-5.9 mg/kg) every 4 hours, however, oral dosages up to 15 mg/kg every 4 hours have been used for short periods (e.g., 4 weeks) in a limited number of patients.

Ribavirin is also contemplated for treatment of RA. This agent has been shown to stimulate T cells (T-lymphocytes) and to produce a dose-dependent inhibition of antigen-induced proliferation. In addition, ribavirin exhibits antiviral activity against many RNA viruses, including Retroviridae, and the subfamily, HTLV-III.

Ribavirin is administered as a solution by nasal and oral inhalation only, using the Viratek small-particle aerosol generator (SPAG) Model SPAG-2. 6 g of ribavirin as a sterile powder is reconstituted by dissolvin it in 50-100 mL of sterile water This solution is then transferred to a sterile 500-mL flask. The flask acts as the reservoir for the SPAG-2 aerosol generator. The solution is further diluted with sterile water for inhalation to a final volume of 300 mL to provide a solution containing 20 mg/mL. Diluents which contain an additive are not usable for the

reconstitution of ribavirin. In addition, the ribavirin solution should not contain any particulate matter and discoloration before its administration.

Once the solution is properly reconstitututed ribavirin solution is obtained, nebulization is preferably administered from the SPAG-2 aerosol generator via an oxygen hood, a face mask or oxygen tent.

When a 20-mg/mL ribavirin solution is used as the starting solution in the SPAG-2 reservoir, the SPAG-2 aerosol generator delivers a mist containing about 190 ug of ribavirin per L. The recommended administration of the ribavirin dose is delivered to the respiratory tract according to the following formula:

respiratory tract = minute volume x time of inhalation x dose (liters) (minutes)
0.19 mg/L (nebulized x 0.7 (fraction of inhaled dose ribavirin concentration) deposited in respiratory tract)

More specifically, at a rate of 12.5 L of mist per minute inhalation therapy with the drug was administered for 16-18 hours. Administration was then continued for 12 hours daily (three 4-hour periods per day) on the second and third days of therapy and for 4 hours on the fourth (final) day of therapy.

Acyclovir is another candidate for treatment of RA. This agent, in parenteral acyclovir therapy, seemed in immunocompromised individuals to lessen inflamation and pain, prevent new lesions, and promote and healing of lesions. In addition, acyclovir's principal therapeutic effect appears to be in preventing the rheumatoid arthritis progression. Although the use of

oral acyclovir has been only recently used, it appears that it may 1) prevent new lesions, 2) possibly decrease the duration of pain, and 3) effect healing of lesions.

In the administration of acyclovir sodium slow intravenous infusion is required. Administration by rapid intravenous infusion (in less than 10 minutes) or rapid intravenous injection is not to be undertaken.

To reconstitute acyclovir sodium, 500 mg of it is added to 10 mL of sterile water for injection. Alternatively, 500 mg of acyclovir sodium is added to bacteriostatic water containing no parabens for injection containing benzyl alcohol. These reconstituted solutions should be used within 12 hours. Prior to using a parenteral dose of acyclovir, the solution must be shaken to completely dissolve the drug. In general, the requisite dose of the reconstituted solution is diluted with 50-125 mL of a compatible intravenous infusion solution. Slow intravenous infusion using diluted solutions generally takes place over a 1-hour period to avoid the risk of adverse renal effects, which can occur if the infusion takes place in less than 1 hour.

An oral dose of 200 mg of acyclovir every 4 hours during waking hours 5 times daily for 10 days is the recommended adult dosage. During recurrent episodes, the recommended adult dosage is 200 mg 3 times daily for up to 26 weeks. In some instances dosages up to 200 mg 5 times daily for up to 26 weeks may be required. Intermittent treatment of recurrent episodes may require a dose of 200 mg every 4 hours while awake 5 times daily for 5 days.

Intravenous dosage of acyclovir for adults with normal renal function (i.e., creatinine clearance greater than 50 mL/minute per 1.73 m^2) is 5 mg/kg every 8 hours (15 mg/kg daily) for 7 to 10 days.

In addition, this invention relates to a method for treating rheumatoid arthritis in a patient comprising administering to the patient an agent which inhibits the attachment of the transformed synovial lining cells to cartilage and bone, thereby inhibiting joint erosion and destruction. The agent is a peptide sequence which binds to the cell attachment receptors.

The present invention is not intended to be limited in scope by the above examples or by the anti-viral agents or treatment regimes described herein, since each is intended merely as an illustration of the invention. In addition, any method of determining whether an RA condition in an individual exists or any method for treating RA with anti-viral agents which are functionally equivalent to those set forth herein is intended to be within the scope of this invention. Indeed various modifications of the invention, in addition to those shown and described herein, will become apparent to those skilled in the art from the foregoing description and accompanying specification. Such modifications are intended to fall within the scope of the appended claims.

I CLAIM

1. A method for diagnosing rheumatoid arthritis in an individual comprising:

a) obtaining a sample from the individual;

b) contacting the sample with an antibody directed against an epitope of a retroviral protein under reaction conditions that permit the antibody to bind the epitope;

c) removing unbound antibody molecules from the sample; and

d) examining the sample for the presence of bound antibody,

wherein the binding of the antibody indicates the presence of rheumatoid arthritis in the individual.

2. The method of claim 1 in which the antibody is labeled.

3. The method of claim 1 which further comprises addition of a labeled specific binding partner to the antibody.

4. The method of claim 1, wherein said antibody is a monoclonal antibody.

5. The method of claim 1, wherein said antibody is selected from the group consisting of anti-HTLV I p19, anti-HTLV I p24, anti-HTLV III p15/17, anti-HTLV III p24, anti-HTLV III gp120 and anti-HTLV reverse transcriptase.

6. The method of claim 1, wherein said retroviral protein is an HTLV protein.

7. The method of claim 6, wherein said HTLV protein is selected from the group consisting of HTLV I p19, HTLV I p24, HTLV III p15/17, HTLV III p24, HTLV III gp120 and anti-HTLV reverse transcriptase.

8. The method of claim 1, wherein said sample is synovial material selected from the group consisting of synovial cells, tissue and fluid.

9. The method of claim 1, wherein said sample are peripheral blood cells.

10. The method of claim 1, wherein step (d) is carried out by an ultrastructural analysis by electron microscopy.

11. The method of claim 10, wherein said ultrastructural analysis by electron microscopy is carried out by immunoelectronmicroscopy.

12. The method of claim 1, wherein step (d) is carried out by an immunofluorescence analysis.

13. The method of claim 1, wherein step (d) is carried out by an immuno-peroxidase technique.

14. The method of claim 1, wherein step (d) is carried out by immunogold staining with silver enhancement.

15. The method of claim 1, wherein step (d) is carried out by immunocytology.

16. The method of claim 1, wherein step (d) is carried out by an immunoblotting.

17. The method of claim 1, wherein step (d) is carried out by an ELISA.

18. A method of diagnosing rheumatoid arthritis in a patient comprising:

(a) isolating a tissue sample from said patient;

(b) contacting said tissue sample of step (a) with a nucleic acid probe specific for a retroviral gene, under conditions that allow hybridization of said probe to nucleic acids in said tissue sample of step (a);

(c) removing said probe of step (b) which is unbound; and

(d) detecting the hybridization of the probe, in which said hybridization of said probe is indicative of rheumatoid arthritis in the patient.

19. The method of claim 18, wherein said retroviral gene of step (b) is an HTLV gene.

20. A method of diagnosing rheumatoid arthritis in a patient comprising:

(a) obtaining a tissue sample from the patient;

(b) isolating a sample of nucleic acid from said tissue sample of step (a);

(c) contacting said sample of nucleic acid of step (b) with a nucleic acid probe specific for a retroviral mRNA sequence, under conditions that allow hybridization of said probe to said isolated sample of nucleic acid of step (b);

(d) removing said probe of step (c) which is unbound; and

(e) detecting said hybridization of said probe of step (c),

in which said hybridization of said probe is indicative of rheumatoid arthritis in the patient.

21. A method of diagnosing rheumatoid arthritis in a patient comprising:

(a) obtaining a tissue sample from the patient;

(b) isolating a sample of nucleic acid from said tissue sample of step (a);

(c) contacting said sample of nucleic acid of step (b) with a nucleic acid probe specific for an retroviral mRNA sequence, under conditions that allow hybridization of said probe to said isolated sample of nucleic acid of step (b);

22. The method of claim 20, wherein said retroviral gene of step (c) is an HTLV gene.

23. The method of claim 21, wherein said retroviral mRNA sequence of step (c) is an HTLV mRNA sequence.

24. A composition for treating a patient having rheumatoid arthritis comprising administration of an effective amount of an anti-viral agent to said patient so as to alleviate said rheumatoid arthritis in said patient.

25. The composition of claim 23, wherein said anti-viral agent is selected from the group consisting of acyclovir, phosphonoformate, ribavirin, "anti-sense" oligodeoxynucleotide, protease inhibitor, 2',3'-dideoxynucleoside, and methylphosphonate oligonucleotide.

26. The composition of claim 25, wherein said 2',3'-dideoxynucleoside is selected from the group consisting of 3'-azido-2',3'-dideoxythymidine and 2',3'-dideoxycytidine.

27. The composition of claim 26, wherein said 3'-azido-2,'3'-dideoxythymidine is administered at a dose of about 2 mg of said 3'-azido-2,'3'-dideoxythymidine to about 15 mg of said 3'-azido-2,'3'-dideoxythymidine/Kg of weight of said patient.

28. A composition for treating rheumatoid arthritis in a patient comprising an effective amount of an agent capable of inhibiting the attachment of the transformed synovial lining cells to cartilage and bone, thereby inhibiting joint erosion and destruction.

29. The composition of claim 28, wherein said agent is a peptide sequence which binds to the cell attachment receptors.

FIG. 1

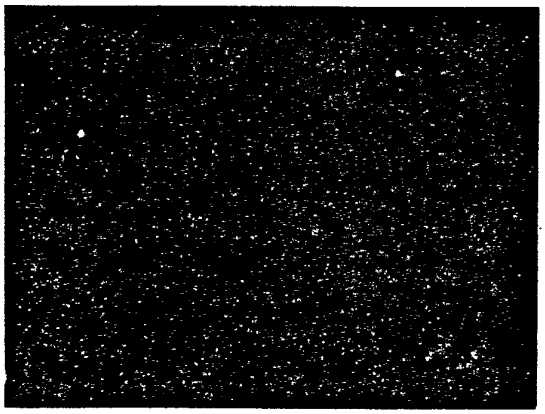

FIG. 2

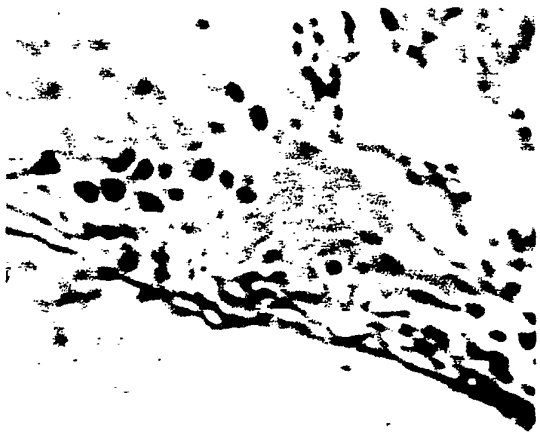

FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No PCT/US89/02219

I CLASSIFICATION OF SUBJECT MATTER

According to International Patent Classification (IPC) or to both National Classification and IPC

See Attachment

II FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
	See Attachment

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched 8

See Attachment

III DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
A,P	EP, A, 0,308,066 (HEM RESEARCH INC.) 22 March 1989, See page 2, line 48- page 3, line 1.	25
X Y	Annals of the Rheumatic Diseases, vol. 44, no. 11, issued Nov. 1985, E. RODAHL ET AL, "Antigens related to the major internal protein, p 27, of a psoriasis associated retrovirus-like particle are expressed in patients with chronic arthritis", pages 761-765. See Abstract.	1,3,8,9,12,15 2,4,10,11,13 14,16-18,20,21
Y	L.E. HOOD ET AL. Immunology, 2nd Ed. 1984, Benjamin Cummings Publishing Co., Inc., Menlo Park, CA, U.S.A. See pages 66-68.	2,10,11,13

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"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)

"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

"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

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"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.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28 July 1989	13 OCT 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	David A. Saunders

Form PCT/ISA/210 (second sheet) (Rev.11-87)

Attachment to Form PCT/IPEA/210 second sheet
I Classification

US.CL. 435/5-7, 28: 436/506,525,530, 801, 805; 514/2, 44-51
Int. Cl.(4) A61K 31/70, 37/02; C12Q 1/68, 70;GOIN 33/548,553,564

II Fields searched

U.S. 435/5-7, 28; 436/506,525, 530,801,805; 514/2, 44-51
Int. Cl. A61K 31/70

Medline: Rheumatoid (w) arthritis and (retrovir? or HTLV or LAV or HIV)

Medline and Biosis: arthriti? and synovi (2w) cell? and (attach? or adher? or adhesi?) and receptor?

APS search: 1) rheumatoid and retrovir? 2) rheumatoid and synovial and (adher? or adheri? or attach?) and (peptide? or polypeptide?)

III DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y,P	US,A 4,775,636 (MOEREMANS ET AL) 04 October 1988. See col. 1 line 48-col. 2, line 16; col. 2, lines 35-50; col.3, lines 47-61, col 6, lines 48-55	2,10,11,13 14,16,17
Y	EP,A 0,185,444 (CENTOCOR, INC) 25 June 1986. See page 5, lines 10-13 and 19-25, page 21, lines 1-9	18,20,21
A	Annals of the Rheumatic Diseases, vol. 47, no.3, issued March 1988, B.K. PELTON ET AL, "A search for retrovirus infection in systemic lupus erythematosus and rheumatoid arthritis", pages 206-209, See Summary; page 206,col.1; and page 209, col 1.	18-23
X	Proceedings of the National Academy of Sciences USA, vol. 82, no. 20 issued October 1985, H. MITSUYA ET AL, "3'-Azido -3'- deoxythymidine (BW A509U): An antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphoadenopathy-associated virus in vitro", pages 7096-7100. See Abstract and Discussion.	24-27

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1 ☐ Claim numbers because they relate to subject matter [2] not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [3], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

See Attachment

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

telephone practice

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ATTACHMENT TO FORM PCT/ 210 SUPPLEMENTAL SHEET

I Claims 1-17 drawn to immunoassays; class 435, subclass 7.

II Claims 18-32, drawn to hybridization assays; class 435, subclass 6.

III Claims 24-27, drawn to compositions of antiviral agents; class 514, subclasses 44+.

IV Claims 28-29, drawn to compositions for inhibiting cell attachment; class 514, subclass 2.

The inventions listed as Group I-IV do not meet the requirement for Unity of Invention for the following reasons: The assays of Groups I and II use no common reagents. The compositions of Group III and IV have no common members and can be used independently in treating arthritis. The compositions of group III can be used to treat conditions other than arthritis. The compositions of groups III and IV can be use to treat arthritis which has been diagnosed by methods other than those of Group I or II.