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(54) Title: DIAGNOSIS AND TREATMENT OF HEPATIC INFLAMMATORY DISORDERS BY INHIBITING THE BINDING OF LFA-1 TO ICAM-1

(57) Abstract: The present invention encompasses methods and compositions useful in diagnosing and treating hepatic disorders, especially those characterized by inflammation. The method comprises administration of an agent which prevents the interaction of ICAM with a ICAM binding partner or ligand. These compositions are useful in treating diseases or disorders involving LFA-I/ICAM blockade, as well as inhibiting a primary event in the inflammatory response such as blocking interactions between intercellular adhesion molecules and their ligands. Disorders treatable using the methods disclosed herein include infections, especially viral infections, iatrogenic disorders, cholestatic disorders, hereditary disorders, sarcoidosis, organ transplant, and the like. The diagnostic methods of the ivention can be employed to detect the presence of a disorder or to monitor the course of therapy used to treat the disorder.

DIAGNOSIS AND TREATMENT OF HEPATIC INFLAMMATORY DISORDERS BY INHIBITING THE BINDING OF LFA-1 TO ICAM-1

Field of the Invention

This invention relates to methods and compositions which can be employed in the prophylaxis, treatment and management of liver disorders, especially those characterized by inflammation. Also disclosed are methods and reagents useful in the prognosis and diagnosis of inflammatory liver disorders.

Description of Related Disclosures

The recruitment and recirculation of leukocytes from blood, through tissue, into lymph and back to the blood are key events in the inflammatory process associated with tissue injury, infection or antigen deposition (Springer, T., (1994) Cell, 76:301-314; Berlin *et al.*, (1995) Cell, 80:413-422; Schweighoffer *et al.*, (1993) J. Immunol., 151:717-729). These events are regulated at a molecular level by interactions between various specialized molecules on the surface of circulating leukocytes and vascular endothelial cells (Springer, T., (1994) supra). Recent models indicate that the recruitment or homing of peripheral blood leukocytes, including lymphocyte subsets, to various tissue sites proceeds by a multistep process comprising i) a primary transient contact event, ii) a rapid activating event that involves G protein-linked signaling receptors and iii) activation triggered firm adhesion (Springer *et al.*, (1994) supra). Chemotaxis and extravasation of leukocytes into tissue follows these three primary events.

Lymphocyte function-associated antigen 1 (LFA-1; CD11a/CD18) is involved in leukocyte adhesion during cellular interactions essential for immunologic responses and inflammation (Larson *et al., Immunol. Rev.* 114:181-217 (1990)). LFA-1 is a member of the β2 integrin family and consists of a unique α subunit, CD11a, and a β subunit, CD18, common to other β2 integrin receptors Mac-1 and p150,95. The ligands of LFA-1 include intercellular adhesion molecule-1, ICAM-1, expressed on leukocytes, endothelium, and dermal fibroblasts (Dustin *et al., J. Immunol.* 137:245-254 (1986)), ICAM-2 expressed on resting endothelium and lymphocytes (de Fougerolles *et al., J. Exp. Med.* 174:253-267 (1991)), and ICAM-3 expressed on monocytes and resting lymphocytes (de Fougerolles *et al., J. Exp. Med.* 179:619-629 (1994)).

Monoclonal antibodies (MAbs) against LFA-1 and the ICAMs have been shown, *in vitro*, to inhibit several T cell-dependent immune functions including T cell activation (Kuypers *et al., Res. Immunol.* 140:461(1989)), T cell-dependent B cell proliferation (Fischer *et al., J. Immunol.* 136:3198-3203 (1986)), target cell lysis (Krensky *et al., J. Immunol.* 131:611-616 (1983)), and adhesion of T cells to vascular endothelium (Lo *et al., J. Immunol.* 143:3325-3329 (1989)). In mice, anti-CD11a MAbs induce tolerance to protein antigens (Tanaka *et al., Eur. J. Immunol.* 25:1555-1558 (1995)) and prolong survival of cardiac (Cavazzana-Calvo *et al., Transplantation* 59:1576-1582 (1995); Nakakura *et al., Transplantation* 55:412-417 (1993)), bone marrow (Cavazzana-Calvo *et al., Transplantation* 59:1576-1582 (1995); van Dijken *et al., Transplantation* 49:882-886 (1990)), corneal (He *et al., Invest. Opthamol. Vis. Sci.* 35:3218-3225 (1994)), islet (Nishihara *et al., Transplantation Proc.* 27:372 (1995)) and thyroid (Talento *et al., Transplantation* 55:418-422 (1993)) allografts.

In humans, anti-CD11a MAbs prevent graft failure after bone marrow transplantation (Fischer *et al., Blood* 77:249-256 (1991); Stoppa *et al., Transplant Intl.* 4:3-7 (1991)) and preliminary clinical

studies of renal allografts treated prophylactically with anti-CD11a MAb, in addition to corticosteroids and azathioprine, are promising (Hourmant *et al., Transplantation* 58:377-380 (1994)). Current therapies against graft rejection include use of OKT3, a murine anti-human CD3 MAb, and cyclosporin A. OKT3 therapy is effective but has several undesirable side effects; its use results in the release of numerous cytokines including tumor necrosis factor-α, interferon-γ, interleukin-2, and interleukin-6, resulting in fever, chills and gastrointestinal distress (for a review see Parlevliet *et al., Transplant Intl.* 5:234-246 (1992); Dantal *et al., Curr. Opin. Immunol.* 3:740-747 (1991)). Cyclosporin A is effective but also has serious side effects (for a review see Barry, *Drugs*, 44:554-566 (1992)).

Summary of the Invention

The present invention encompasses methods and compositions useful in the diagnosis, prognosis and treatment of hepatic disorders. The methods and compositions of the invention can be employed in the diagnosis, prognosis and treatment of a variety of hepatic disorders, especially those characterized by ICAM associated leukocyte recruitment to the liver. Hepatic disorders within the present invention include any disease or disorder characterized by ICAM expression including infections, especially viral infections, autoimmune disorders, iatrogenic disorders, hereditary disorders, cholestatic disorders, sarcoidosis, liver injury as a result of organ transplantation, i.e., in graft rejection or graft versus host disease such as after bone marrow transplant. The invention is preferably used to treat hepatitis, especially viral hepatitis, drug induced hepatitis, and autoimmune hepatitis, cholestatic disorders such as primary biliary cirrhosis and primary sclerosing cholangitis and allograft rejection.

The methods of treatment encompassed within the present invention comprise administration to a host in need thereof of an agent which prevents the interaction of ICAM with a ICAM binding partner or ligand such as LFA-1 or which inhibits the expression of ICAM in the liver. Hepatic leukocytes, for example, lymphocytes, may be resident or recruited to the liver. The methods are useful in preventing ICAM associated leukocyte recruitment to the liver as well as inhibiting a primary event in the inflammatory response such as blocking interactions between intercellular adhesion molecules and their ligands. They are also useful in preventing ICAM associated activation of resident leukocytes within the liver. In preferred embodiments, the methods of the present invention are employed to reduce or prevent the infiltration of LFA-1 bearing leukocytes into liver thereby decreasing the severity of inflammation and the degree of tissue injury in the hepatic disease or disorder treated. Severity of inflammation and degree of tissue damage will also be blocked or reduced by preventing ICAM associated activation of resident hepatic leukocytes.

Another aspect is a method of inhibiting the binding between a first cell expressing a ligand for ICAM and a second cell expressing ICAM, wherein the second cell is present in a liver, comprising contacting one or both of the cells *in vivo* or *in vitro* with an agent which prevents the interaction of ICAM with the ICAM ligand. In one embodiment, the ligand for ICAM is LFA-1 and the agent used to block the interaction is an anti-LFA-1 antibody, preferably an anti-CD11a antibody, preferably a human or humanized form of the antibody.

The invention includes compositions, including pharmaceutical compositions comprising agents such as antibodies for the treatment of hepatic disorders as well as kits and articles of manufacture. Kits and articles of manufacture preferably include:

(a) a container;

(b) a label on said container; and

(c) a composition comprising an active agent contained within said container; wherein the composition is effective for treating a hepatic disorder, the label on said container indicates that the composition can be used for treating a hepatic disorder, and the active agent in said composition comprises an agent which prevents the interaction of ICAM with a ligand therefor. The kits optionally include accessory components such as a second container comprising a pharmaceutically-acceptable buffer and instructions for using the composition to treat a hepatic disorder.

Also disclosed are methods useful in the prognosis and diagnosis of hepatic disorders, especially those characterized by the expression of ICAM and/or LFA-1 in hepatic tissue. The diseases or disorders for prognosis or diagnosis under the present invention include those diseases and disorders treatable within the context of the present invention. The diagnostic methods can be employed to detect the presence of ICAM in a sample, especially a liver biopsy or the presence of infiltrating leukocytes bearing a ligand for ICAM in the sample. The methods can be employed to detect the disorder or to monitor, stage or predict the course of the disease or the therapy used to treat the disorder.

Brief Description of the Figures

Figure 1 shows that treatment with anti-CD11a significantly reduces the degree of portal inflammation, when compared with isotype treated controls. Statistical analysis was with Scheffe's test and graphs show means ± 1 S.E.

Figure 2 shows that treatment with anti-CD11a significantly reduces the severity of the total hepatitis score, when compared with isotype treated controls. Statistical analysis was with Scheffe's test and graphs show means ± 1 S.E.

Detailed Description of the Preferred Embodiments

Definitions

In general, the following words or phrases have the indicated definition when used in the description, examples, and claims.

"ICAM" in the context of the present invention refers to the protein intercellular adhesion molecules, ICAM-1 expressed on leukocytes, endothelium, and dermal fibroblasts (Dustin *et al., J. Immunol.* 137:245-254 (1986)), ICAM-2 expressed on resting endothelium and lymphocytes (de Fougerolles *et al., J. Exp. Med.* 174:253-267 (1991)), and ICAM-3 expressed on monocytes and resting lymphocytes (de Fougerolles *et al., J. Exp. Med.* 179:619-629 (1994)).

By "ICAM-binding partner or ligand" it is meant a molecule that interacts with ICAM. The molecule may be naturally occurring and may be soluble or localized to the surface of a cell. One known ICAM ligand is the lymphocyte integrin LFA-1 ($\alpha L\beta 2$ or CD11a/CD18), a heterodimeric structure consisting of an α and a β subunit.

A "conditioning dose" is a dose which attenuates or reduces the frequency or the severity of first dose adverse side effects associated with administration of a therapeutic compound. The conditioning dose may be a therapeutic dose, a sub-therapeutic dose, a symptomatic dose or a sub-symptomatic dose. A therapeutic dose is a dose which exhibits a therapeutic effect on the patient and a sub-therapeutic dose is a dose which dose not exhibit a therapeutic effect on the patient treated. A symptomatic dose is a dose which induces at least one adverse effect on administration and a sub-symptomatic dose is a dose which does not induce an adverse effect.

A "therapeutically effective amount" refers to the minimum concentration (amount) of an agent herein administered to a mammal that is effective in at least attenuating a pathological symptom (*e.g.* causing, inducing or resulting in a detectable / measurable improvement; lessen the severity, extent or duration of symptoms) which occurs as a result of a hepatic disorder.

"Treatment" is an intervention performed with the intention of preventing the development or altering the pathology of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent, slow down or lessen the severity, extent or duration of symptoms, or delay the onset of (e.g., in subjects predisposed to develop a hepatic disorder due to genetic make-up or other risk factors, e.g., in cirrhosis) a targeted pathological condition or disorder. Thus, for example, the term treatment includes the administration of an agent prior to or following the onset of a disease or disorder or after clinical manifestation of the disease thereby preventing or removing all signs of the disease or disorder. "Treating" a disease, disorder, condition or cell population includes therapy and prophylactic treatment on an acute short term basis and on a chronic long-term basis. Treatment is successful if it results in a detectable or measurable improvement in at least one symptom of the disorder being treated (consistent with the definition of "therapeutically effective amount" above). Thus, administration of the agent after onset and after clinical symptoms have developed where administration affects clinical parameters of the disease or disorder, such as the degree of tissue injury or the amount or extent of leukocyte trafficking and perhaps amelioration of the disease, comprises "treatment" of the disease. The diagnosis of hepatic disorders, symptoms, clinical parameters, is described, e.g., in Pathology of the Liver, 3rd. Edition, (MacSween, Anthony, Scheuer, Burt and Portman, eds.) Churchill Livingstone (1994) the disclosure of which is incorporated in its entirety herein by reference, and will be familiar to the physician of skill in the art. Grading hepatitis is also exemplified in the working examples below.

Those "in need of treatment" include mammals, such as humans, already having the disease or disorder, including those in which the disease or disorder is to be prevented.

The expressions, "agent", "composition" and "antagonist" are used within the scope of the present invention interchangeably and are meant to include any molecule or substance which prevents the interaction between ICAM and a ICAM ligand or binding partner, such as LFA-1. Such molecules include small bioorganic molecules, e.g. peptidomimetics, antibodies, immunoadhesins, proteins, peptides, glycoproteins, glycopeptides, glycolipids, polysaccharides, oligosaccharides, nucleic acids, bioorganic molecules, pharmacological agents and their metabolites, transcriptional and translation control sequences, and the like.

The term "inflammation" is used here to refer to reactions of both the specific and non-specific defense systems. A specific defense system reaction is a specific immune system reaction to an antigen. Examples of specific defense system reactions include T cell and antibody response to antigens, such as viruses, and delayed-type hypersensitivity. A non-specific defense system reaction is an inflammatory response mediated by leukocytes generally incapable of immunological memory. Such cells include macrophages, eosinophils and neutrophils. Examples of non-specific reactions include the immediate swelling after a bee sting, and the collection of PMN leukocytes at sites of bacterial infection, e.g., pulmonary infiltrates in bacterial pneumonias and pus formation in abscesses.

The term "iatrogenic disorder" refers to those disorders induced by exposure to a therapeutic compound or surgical treatment intended to treat some other disorder. Examples of drug induced liver diseases or disorders include, for example chronic active hepatitis associated with administration of Amineptine, Clometacine, Dantrolene, Diclofenac, Fenofibrate, Triglitazone, Piaglitazone, to name but a few; chronic cholestasis associated with the administration of Aceprometazine, Ajmaline and related drugs, Amitryptyline, and Ampicillin to name but a few; or hepatic granulomas associated with administration of Allopurinal, Aspirin, and Diazepam to name but a few. In this context reference can be made to Tables 15.10, 15.8 and 15.11 of Pathology of the Liver, 3rd. Edition, (Macsween, Anthony, Scheuer, Burt and Portman, eds.) Churchill Livingstone (1994) the disclosure of which is incorporated in its entirety herein by reference.

The term "antibody" is used in the broadest sense and specifically covers single monoclonal antibodies (including agonist and antagonist antibodies) and antibody compositions with polyepitopic specificity.

The term "monoclonal antibody" (mAb) as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each mAb is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they can be synthesized by hybridoma culture, uncontaminated by other immunoglobulins.

The monoclonal antibodies herein include hybrid and recombinant antibodies produced by splicing a variable (including hypervariable) domain of one antibody with a constant domain of another antibody, or a light chain with a heavy chain, or a chain from one species with a chain from another species, or fusions with heterologous proteins, regardless of species of origin or immunoglobulin class or subclass designation, as well as antibody fragments (e.g., Fab, $F(ab')_2$, and Fv), so long as they exhibit the desired biological activity. (See, e.g., Cabilly *et al.*, U.S. Pat. No. 4,816,567; Mage and Lamoyi, in Monoclonal Antibody Production Techniques and Applications, pp. 79-97 (Marcel Dekker, Inc., New York, 1987).)

Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of

the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention can be made by the hybridoma method first described by Kohler and Milstein, Nature 256:495 (1975), or can be made by recombinant DNA methods (Cabilly *et al., supra*).

The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; and Morrison *et al., Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). Chimeric antibodies of interest herein include "primatized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (*e.g.* Old World Monkey, Ape etc) and human constant region sequences.

"Humanized" forms of non-human (e.g., murine) antibodies are specific chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$, or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies can comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences of the donor antibody. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details see Jones *et al.*, Nature 321:522 (1986); Reichmann *et al.*, Nature 332:323 (1988); and Presta, Curr. Op. Struct. Biol. 2:593 (1992).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (*e.g.* residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al., Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (*e.g.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). "Framework Region" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the "binding domain" of a heterologous protein, for example ICAM (an "adhesin", for example, a receptor, ligand or enzyme) with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of the adhesin amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site (antigen combining site) of an antibody (i.e. is "heterologous") and an immunoglobulin constant domain sequence. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG, IgM, IgE, IgA, and any subclass or isotype thereof.

The term "anti-LFA-1 antibody" or "anti-LFA-1 MAb" refers to an antibody directed against either CD11a or CD18 or both. The anti-CD11a antibodies include, e.g., MHM24 [Hildreth *et al.*, Eur. J. Immunol., 13: 202-208 (1983)], humanized MHM24 also referred to as hu1124 (described in U.S. 6,037,454), R3.1 (IgG1) [R. Rothlein, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT], 25-3 (or 25.3), an IgG1 available from Immunotech, France [Olive *et al.*, in Feldmann, ed., Human T cell Clones. A new Approach to Immune Regulation, Clifton, NJ, Humana, 1986 p. 173], KBA (IgG2a) [Nishimura *et al.*, Cell. Immunol., 107: 32 (1987); Nishimura *et al.*, *ibid.*, 94: 122 (1985)], M7/15 (IgG2b) [Springer *et al.*, Immunol. Rev., 68: 171 (1982)], IOT16 [Vermot Desroches *et al.*, Scand. J. Immunol., 33: 277-286 (1991)], SPVL7 [Vermot Desroches *et al.*, *supra*], M17 (IgG2a), available from ATCC, which are rat anti-murine CD11a antibodies, and the antibodies described in US 5,622,700, WO 93/21953, US 5,730,983, and US 5,597,567, for example.

Examples of anti-CD18 antibodies include MHM23 [Hildreth *et al.*, *supra*], M18/2 (IgG2a) [Sanches-Madrid *et al.*, J. Exp. Med., 158: 586 (1983)], H52 [Fekete *et al.*, J. Clin. Lab Immunol., 31: 145-149 (1990)], Mas191c [Vermot Desroches *et al.*, *supra*], IOT18 [Vermot Desroches *et al.*, *supra*], 60.3 [Taylor *et al.*, Clin. Exp. Immunol., 71: 324-328 (1988)], 60.1 [Campana *et al.*, Eur. J. Immunol., 16: 537-542 (1986)] and the antibodies described in WO 94/02175, US 5,324,510, US 5,310,551, WO 15076, US 5,597,5657, US 5,437,958, US 5,470,953, US 5,854,070, US 5,914,112, EP 438312, US 5,147,637, WO 93/02191 and WO 94/12214, for example.

Other examples of suitable LFA-1 antagonists, including antibodies, are described in Hutchings *et al.*, *supra*, WO 91/18011 published Nov. 28, 1991, WO 91/16928 published Nov. 14, 1991, WO 91/16927 published Nov. 14, 1991, Can. Pat. Appln. 2,008,368 published June 13, 1991, WO 90/15076

published Dec. 13, 1990, WO 90/10652 published Sept. 20, 1990, EP 387,668 published Sept. 19, 1990, EP 379,904 published Aug. 1, 1990, EP 346,078 published Dec. 13, 1989, U.S. Pat. No. 5,071,964, U.S. Pat. No. 5,002,869, Australian Pat. Appln. 8815518 published Nov. 10, 1988, EP 289,949 published Nov. 9, 1988, and EP 303,692 published Feb. 22, 1989.

Examples of ICAM-1 antibodies include YN/1.7.4; RR1/1 (Rothlein, R. et al., J. Immunol. 137:1270-1274 (1986) and R6-5-D6 and R6.5 "enlimomab" (U.S. patents 5,284,931 and 5,475,091; Rothlein, R., et al., J Immunol. 141:1665-1669 (1988)); LB-2 (Clark, E.A. et al., In: Leukocyte Typing / (A. Bernard, et al., Eds.), Springer- Verlag pp 339-346 (1984)); and CL203 (Staunton, D.E. et al., Cell 56:849-853 (1989)). ICAM-2 antibodies are described in U.S. 5,565,550. Soluble fragments of human ICAM-1 are described, e.g., in U.S. 5,831,036. These references describe how to prepare ICAM antibodies.

Detailed Description of the Preferred Embodiments

Hepatic Diseases and Disorders

The methods of the invention are useful in the diagnosis, prognosis and treatment of a variety of hepatic disorders, in particular those characterized by the presence of ICAM bearing cells. Therefore, according to the present invention, a hepatic disorder or disease is any liver disease or disorder accompanied by the expression of ICAM in the liver and surrounding vasculature. For example, the methods of the invention are useful in the diagnosis, prognosis and treatment of variety of hepatic disorders including those resulting from infection, iatrogenic disorders, hereditary disorders, autoimmmune disorders, cholestatic syndromes, sarcoidosis, organ transplantation, and the like so long as the disorder is characterized by the presence of ICAM bearing cell types.

Diseases or disorders within the scope of the present invention include but are not limited to the diseases and disorders detailed in Table I.

TABLE I

Systemic Diseases and Disorders Involving Liver Inflammation

A. Hepatitis

1. Any inflammation of the liver, (e.g. portal, lobular, perivenular) as for example in acute hepatitis, chronic hepatitis, alcoholic hepatitis and cirrhosis.

2. Infection

Any inflammation of the liver resulting from infection, especially viral infection, especially chronic viral hepatitis, for example inflammation associated with:

a) Hepatitis A, picorna virus

b) Hepatitis B, hepadna virus (hepatocellular carcinoma)

c) Hepatitis C, flavivirus

d) Hepatitis D (Δ), incomplete RNA virus (requires co-infection with hepatitis B)

e) Hepatitis E, single stranded, positive sense RNA genome

f) Hepatitis F,

g) Hepatitis G (HGBV-C) single stranded RNA virus

h) Epstein-Barr virus

i) cytelomegalovirus

j) adenovirus

k) other viral infections of the liver

3. Autoimmune

Any inflammation of the liver associated with autoimmune onset of known or unknown etiology, typically associated with significant lymphocyte infiltration in the portal tracts and associated piecemeal necrosis.

4. Iatrogenic

Any drug induced liver inflammation, including for example, chronic active hepatitis, cholestasis or granuloma formation.

5. Hereditary

Any inflammation associated with gene-linked trait, for example cirrhotic changes in the liver associated with hepatolenticular degeneration,

a) Wilson's disease

b) α-1-antitrypsin deficiency

c) other inherited metabolic disorders, for example, galactosemia

B. Cholestatic Syndromes

Any inflammation of the intrahepatic bile ducts, including those resulting in hepatic dysfunction and cirrhosis as for example in primary biliary cirrhosis, primary sclerosing cholangitis and adult idiopathic ductopenia

C. Transplantation

Any inflammation of the liver or hepatic ducts including that associated with hepatic transplantation, liver injury in graft versus host disease and recipients of renal and other allografts, for example hyperacute allograft rejection, and xenograft rejection.

Particularly preferred disorders within the context of the invention are chronic hepatitis particularly hepatitis resulting from infection, particularly viral infection. Included in this category are the established serological categories of chronic hepatitis, including viral (HBV, HDV, HCV), autoimmune hepatitis (classic lupoid type and subtypes), autoimmune overlap syndromes, drug induced (any hepatitis inducing compound, for example, nitrofurantoin, alpha methyldopa, isoniazid) and so-called "cryptogenic" hepatitis. In this regard the skilled artisan will make reference to chapter 9, and especially Tables 9.2 and 9.3 in Pathology of the Liver, 3rd. Edition, (MacSween, Anthony, Scheuer, Burt and Portman, eds.) Churchill Livingstone (1994) the disclosure of which is incorporated in its entirety herein by reference. As the skilled artisan will recognize, some chronic liver diseases not included within the definition of chronic hepatitis may have histological features of chronic hepatitis (for example, piecemeal necrosis). These disorders such as, for example, diseases of intra or extrahepatic bile ducts, are included within the definition herein. Infection with a number of viruses is known to result in serious inflammation of the liver including the hepatitis viruses, hepatitis A (HAV), hepatitis B (HBV), hepatitis C (HCV), hepatitis D (HDV, delta agent) hepatitis E, hepatitis F and other viruses such as Epstein-Barr virus, cytomegalovirus, adenovirus, paramyxovirus, and the like. At least seven types of hepatitis virus (designated A - G) have been identified to date. Of these, one of the most devastating is hepatitis C virus (HCV, also called non-A, non-B). An estimated 3.9 million people in the US are currently infected with HCV, and an estimated 8,000 - 10,000 deaths each year result from HCV-associated chronic liver disease. Current therapies include γ-interferon, empliasize B and ribivirin, each of which have limited efficacy and serious side effects. Current therapy also includes transplantation, however, since the infected individual remains infected with the virus, post-transplant immunosuppressed patients exhibit increased viral RNA levels and often rapidly progress to liver disease with the new liver.

Chronic cholestatic syndromes are characterized by progressive inflammatory destruction of intrahepatic bile ducts resulting in hepatic dysfunction, fibrosis and cirrhosis. Examples of this type of disorder include primary biliary cirrhosis, primary sclerosing cholangitis and adult idiopathic ductopenia.

Hereditary disorders treatable by the methods disclosed herein include those inflammatory disorders associated with a gene-linked trait. Examples include Wilson's disease, α-1-antitrypsin deficiency and inherited metabolic disorders such as galactosemia and tyrosineanemia.

Diagnosing and Prognosing a Hepatic Disorder

Hepatic disorders for prognosis and diagnosis within the context of the present invention are described above and are characterized by the presence of ICAM in a sample, for example a sample of hepatic tissue or surprisingly, in a cell free sample such as serum. Therefore, one embodiment of the present invention is directed to the detection and/or measurement of ICAM in a sample and the use of such detection or measurement in the diagnosis, staging, determination of severity, and prognosis in general of the hepatic disease or disorder. Further, since the expression of ICAM has been shown to correlate with the presence of lymphocytes bearing the LFA-1 integrin, prognosis and diagnosis of hepatic disorders within the context of the present invention encompass the measurement or detection of the presence of lymphocytes bearing LFA-1 integrin.

A. Detecting Soluble or Cell-free ICAM

The present invention includes a method for diagnosis and prognosis of diseases and disorders not limited to hepatic diseases and disorders but appropriately used therefor, based upon the discovery that ICAM can be detected in the serum of a subject. Therefore the present invention includes methods of diagnosis and prognosis of diseases or disorders characterized by the expression of ICAM bearing cell types in general and which include but are not limited to the hepatic diseases or disorders listed above.

According to this aspect of the present invention, a sample which is subjected to testing is a sample derived from a subject such as a human and includes, but is not limited to, any biological fluid, preferably a bodily fluid. Especially preferred are cell-free samples, the term cell-free being used herein to indicate that the sample is substantially devoid of cells or that the sample is substantially free of cell types bearing ICAM. Examples of bodily fluids include, but are not limited to, whole blood, serum, plasma, urine, synovial fluid, cranial or spinal fluid, saliva, tissue infiltrate, cervical or vaginal exudate, tissue infiltrate, pleural effusions, bronchoalveolar lavage fluid, gastric lavage fluid, small or large bowel contents, fecal preparations, and the like. In another embodiment, the biological fluid may be a cell culture medium or supernatant of cultured cells. Preferably the sample is a blood sample and especially a serum sample.

The methods provided by the present invention overcome many of the limitations of prior art methods of measuring or detecting ICAM, which heretofore required samples comprising cells followed by immunohistochemical techniques or direct or indirect immunofluorescence analysis by microscopy or flow cytometry. Limitations of the prior art procedures include the requirement for: (1) fairly rare tissue samples comprising a large number of cells, (2) extensive preparation time, and (3) expensive equipment, such as a flow cytometer. The methods provided herein overcome these limitations.

Any procedure known in the art for the measurement of analytes can be used in the practice of the measurement of ICAM in a sample. Such procedures include but are not limited to competitive and non-competitive assay systems using techniques such as radioimmunoassays, enzyme immunoassays (EIA), preferably the enzyme linked immunosorbent assay (ELISA), "sandwich" immunoassays, precipitin reactions, gel diffusion reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A

immunoassays, and immunoelectrophoresis assays, to name but a few. For examples of preferred immunoassay methods, see U.S. Patents No. 4,845,026 (July 4, 1989) and No. 5,006,459 (April 9, 1991).

For diagnostic and prognostic applications, an ICAM binding partner, typically an antibody will be labeled with a detectable moiety and used to detect ICAM in a sample as described above. Numerous labels are available which can be preferably grouped into the following categories:

(a) Radioisotopes, such as ^{35}S, ^{14}C, ^{125}I, ^{3}H, and ^{131}I. The ICAM binding partner such as an antibody can be labeled with the radioisotope using the techniques described in *Current Protocols in Immunology*, Volumes 1 and 2, Coligen *et al.*, Ed., Wiley-Interscience, New York, New York, Pubs., (1991) for example and radioactivity can be measured using scintillation counting.

(b) Fluorescent labels such as rare earth chelates (europium chelates) or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, Lissamine, phycoerythrin and Texas Red are available. The fluorescent labels can be conjugated to the ICAM binding partner such as an antibody using the techniques disclosed in *Current Protocols in Immunology*, *supra*, for example. Fluorescence can be quantified using a fluorimeter.

(c) Various enzyme-substrate labels are available and U.S. Patent No. 4,275,149 provides a review of some of these. The enzyme preferably catalyses a chemical alteration of the chromogenic substrate which can be measured using various techniques. For example, the enzyme may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. Techniques for quantifying a change in fluorescence are described above. The chemiluminescent substrate becomes electronically excited by a chemical reaction and may then emit light which can be measured (using a chemiluminometer, for example) or donates energy to a fluorescent acceptor. Examples of enzymatic labels include luciferases (*e.g.*, firefly luciferase and bacterial luciferase; U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase, -galactosidase, glucoamylase, lysozyme, saccharide oxidases (*e.g.*, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating enzymes to antibodies are described in O'Sullivan *et al.*, Methods for the Preparation of Enzyme-Antibody Conjugates for use in Enzyme Immunoassay, in *Methods in Enzym.* (ed J. Langone & H. Van Vunakis), Academic press, New York, 73: 147-166 (1981).

Examples of enzyme-substrate combinations include, for example:

(i) Horseradish peroxidase (HRPO) with hydrogen peroxidase as a substrate, wherein the hydrogen peroxidase oxidizes a dye precursor (*e.g.* orthophenylene diamine (OPD) or 3,3',5,5'-tetramethyl benzidine hydrochloride (TMB));

(ii) alkaline phosphatase (AP) with para-Nitrophenyl phosphate as chromogenic substrate; and

(iii) β-D-galactosidase (β-D-Gal) with a chromogenic substrate (*e.g.* p-nitrophenyl-β-D-galactosidase) or fluorogenic substrate 4-methylumbelliferyl-β-D-galactosidase.

Numerous other enzyme-substrate combinations are available to those skilled in the art. For a general review of these, see U.S. Patent Nos. 4,275,149 and 4,318,980.

In the assays of the present invention, an ICAM binding partner such as an antibody is preferably bound to a solid phase support or carrier. By "solid phase support or carrier" is intended any support capable of binding an antigen or antibodies. Well-known supports, or carriers, include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

In a preferred embodiment, an antibody-ICAM-antibody sandwich immunoassay is done, *i.e.*, ICAM is detected or measured by a method comprising binding of a first antibody to the ICAM antigen, and binding of a second antibody to the ICAM, and detecting or measuring ICAM immunospecifically bound by both the first and second antibody. In a specific embodiment, the first and second antibodies are monoclonal antibodies. In this embodiment, the second monoclonal antibody preferably binds to a site different from that of the first antibody (as reflected *e.g.*, by the lack of competitive inhibition between the two antibodies for binding to the antigen). In another specific embodiment, the first or second antibody is a polyclonal antibody. In yet another specific embodiment, both the first and second antibodies are polyclonal antibodies.

In a preferred embodiment, a "forward" sandwich enzyme immunoassay is used, as described schematically below. An antibody (capture antibody, Ab1) directed against the ICAM is attached to a solid phase matrix, preferably a microplate. The sample is brought in contact with the Ab1-coated matrix and such that any ICAM in the sample to which Ab1 is specific binds to the solid-phase Ab1. Unbound sample components are removed by washing. An enzyme-conjugated second antibody (detection antibody, Ab2) directed against a second epitope of the ICAM binds to the antigen captured by Ab1 and completes the sandwich. After removal of unbound Ab2 by washing, a chromogenic substrate for the enzyme is added, and a colored product is formed in proportion to the amount of enzyme present in the sandwich, which reflects the amount of ICAM in the sample. The reaction is terminated by addition of stop solution. The color is measured as absorbance at an appropriate wavelength using a spectrophotometer. A standard curve is prepared from known concentrations of the ICAM, from which unknown sample values can be determined.

Other types of "sandwich" assays are the so-called "simultaneous" and "reverse" assays. A simultaneous assay involves a single incubation step as the antibody bound to the solid support and labeled antibody are both added to the sample being tested at the same time. After the incubation is completed, the solid support is washed to remove the residue of fluid sample and uncomplexed labeled antibody. The presence of labeled antibody associated with the solid support is then determined as it would be in a conventional "forward" sandwich assay.

In the "reverse" assay, stepwise addition first of a solution of labeled antibody to the fluid sample followed by the addition of unlabeled antibody bound to a solid support after a suitable incubation period is utilized. After a second incubation, the solid phase is washed in conventional fashion to free it of the residue of the sample being tested and the solution of unreacted labeled antibody. The determination of labeled antibody associated with a solid support is then determined as in the "simultaneous" and "forward" assays.

B. Diagnostic and Prognostic Methods in General

The methods of the present invention can be used alone or in conjunction with other diagnostic tests for the diagnosis and detection of a hepatic disorder. Viral infections can be detected using techniques known in the art. Hepatitis C infection, for example, can be detected using commercially available serologic assays which detect anti-HCV antibodies or molecular assays which detect HCV RNA genomes within an infected patient. The methods of present invention can be used alone or in conjunction with these routine tests as an aid in diagnosis. As another example, in many cases the specific cause of a liver disorder is identified on the basis of elevated liver function tests or an enlarged liver. The diagnostic methods of the present invention can be used alone or in conjunction with these tests to diagnose a disease or disorder within the context of the present invention. As but a further example, blood tests and a liver biopsy are routinely used to diagnose or confirm a diagnosis and as well to determine the amount, extent and severity of damage to the liver. The diagnostic methods of the present invention can be used alone or in conjunction with these tests in determining the amount, extent, or severity of damage to the liver.

Somewhat more particularly, a diagnostic test performed on for example a serum sample, an *in vivo* sample or a liver biopsy can, with the present invention, be extended to the detection of ICAM or LFA-1 expression in the sample. Further, the detection of ICAM or LFA-1 in the sample can be used to monitor the course or progression of the disease as well as the course of or effectiveness of a therapeutic treatment.

In a particular embodiment, the diagnostic techniques described can be used to follow the progress of therapy. In a subject undergoing therapeutic treatment that results in an increase or a decrease in the amount of trafficking lymphocytes, the amount of lymphocyte trafficking may serve as a useful measure for the success or failure of the treatment. Thus, the present invention provides a method for monitoring the effect of a therapeutic treatment in a subject which comprises measuring at suitable time intervals the amount of ICAM expressed in a sample of liver tissue or conversely the amount or number of lymphocytes in the sample. The total amount of ICAM or LFA-1 is compared to a "baseline" or "control" value which depending on the disease, and the treatment, may be the amount of ICAM in a similar sample from a normal subject, from the patient prior to disease onset or during remission of disease, or from the patient prior to the initiation of therapy. One of ordinary skill in the art will readily discern the appropriate baseline value to use in a particular situation without undue experimentation.

A preferred subject for the methods of the present invention is a vertebrate, including but not limited to a mammal, fish, amphibian, reptile, bird, marsupial, and most preferably, a human either fetal

or adult human liver. Thus the methods and kits of this invention are applicable to human clinical and veterinary uses.

According to a particular aspect of the present invention, a sample, for example a liver biopsy sample is derived from a subject by methods routine to those skilled in the art. The most common way a liver sample is obtained is by liver biopsy, a procedure used to obtain a small amount of liver tissue which can be subsequently examined employing routine immunohistochemical techniques in conjunction with the methods of the present invention. For example liver sample can be obtained by needle biopsy directly into the liver of subject, or for example by guiding a needle into the liver of the subject through the abdomen or chest using various imaging techniques known to the skilled artisan. Less commonly, samples are obtained using techniques such as laproscopy, transvenous or transjugular liver biopsy and surgical liver biopsy.

As noted above any procedure known in the art for the measurement of analytes can be used in the practice of the instant invention to detect the presence of ICAM or a ligand therefor, such as LFA-1. Such procedures include but are not limited to immunohistochemical techniques known to those skilled in the art, competitive and noncompetitive assay systems using techniques such as radioimmunoassays, enzyme immunoassays (EIA), preferably the enzyme linked immunosorbent assay (ELISA), "sandwich" immunoassays, precipitin reactions, gel diffusion reactions, immunodiffusion assays, agglutination assays, complement fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, and immunoelectrophoresis assays, to name but a few.

Kits comprising one or more containers or vials containing components for carrying out the assays of the present invention are also within the scope of the invention. For instance, such a kit can comprise reagents required for the immunohistochemical analysis of a sample such as a liver biopsy. Reagents may include one or more binding partners, *e.g.*, an antibody or antibodies, to antigen, for example a leukocyte integrin or other ICAM binding partner, or ICAM itself. For histological assays the kit contains the chromogenic substrate as well as a reagent for stopping the enzymatic reaction when color development has occurred. The substrate included in the kit is one appropriate for the enzyme conjugated to one of the antibody preparations such as an anti-human ICAM antibody. These are well-known in the art. The kit can optionally also comprise a standard; *i.e.*, a known amount of purified ICAM.

In another embodiment, a kit can comprise more than one set of reagents. For example a kit can comprise a pair of antibodies or other binding partners, each pair directed against a different target molecule, thus allowing the detection or measurement of a plurality of such target molecules in a sample, for example, ICAM and a liver cell-specific surface protein or ICAM and LFA-1.

Compositions

Compositions useful in the therapeutic and the diagnostic methods of the present invention, are available to the skilled artisan and can be identified based upon their ability to prevent, block or suppress ICAM mediated cell adhesion. The compositions are useful in the treatment and diagnosis of hepatic disorders associated with that adhesion, such as inflammation and immune reactions.

It will be understood that appropriate agents able to prevent, block or suppress ICAM mediated cell adhesion may accomplish this effect in various ways. Without limitation to a particular theory, one class of agents will bind to ICAM with sufficient affinity and specificity to prevent interaction with lymphocytes expressing a naturally occurring ligand for ICAM, such as the lymphocyte integrin LFA-1. Another class of agents will bind to a naturally occurring leukocyte ligand for ICAM, such as the lymphocyte integrin LFA-1 and thereby prevent its interaction with ICAM.

Exemplary agents are antibodies preferably a monoclonal, chimeric and or humanized antibody or an antigen binding fragment thereof which inhibits adhesion of leukocytes to ICAM. A further exemplary agent is a soluble ICAM molecule or a molecule based upon ICAM such as a soluble form of ICAM comprising the integrin binding site of ICAM or an ICAM immunoadhesin comprising, for example, the extracellular domain of ICAM fused to an immunoglobulin constant domain.

As a further example of an agent, a peptide or a molecule based upon a peptide sequence present in ICAM and required for integrin binding can be used as an agent within the context of the present invention. Alternatively, it has been shown that integrins can selectively bind a variety of Arg-Gly-Asp (RGD) containing ligands. RGD-based peptide inhibitors with different structures can be prepared which are effective agents within the context of the present invention.

A further agent is an antisense nucleic acid, which is complementary, in whole or in part, to a target molecule comprising a sense strand, and can hybridize to the target molecule. When introduced into a cell antisense nucleic acid can inhibit the expression of the gene encoded by the sense strand. Antisense nucleic acid in whole or in part complimentary to the nucleic acid sequence of ICAM can be produced for this purpose.

In a preferred embodiment the agent is an antibody, which antibody has the desirable properties of binding to ICAM and preventing its interaction with the leukocyte associated ligand or binding to LFA-1 and preventing the interaction of LFA-1 with ICAM. Useful antibodies are available to the skilled artisan such as those described herein or those described in the references identified above in the definitions of anti-LFA-1 antibodies, anti-CD11a antibodies, and anti-CD18 antibodies. The following techniques can, without limitation, be employed in identifying and isolating appropriate agents useful in blocking or preventing the interaction between ICAM and an ICAM binding partner. The compositions of the invention can be assayed by techniques known in the art in order to demonstrate their activity. Such assays include, but are not limited to, the following *in vitro* tests for the ability to interact with ICAM proteins, to inhibit ICAM related activity, or to selectively inhibit the generation of ICAM derived peptides.

In another appropriate assay, purified LFA-1 is immobilized on a solid support such as a glass slide or a plastic plate pre-incubated with an antibody to the α subunit that does not block interaction of the integrin with ICAM. In this assay ICAM or preferably a ICAM-immunoglobulin chimera is incubated with the immobilized integrin in the presence or absence of a suspected agent. The binding or absence of binding of ICAM in the presence of the agent being tested can then be measured with a detecting agent such as an anti-ICAM or anti-Ig antibody.

Alternatively a cell based assay employing a cell transfected with the LFA-1 integrin subunits and which expresses the intact integrin can be used in a cell based assay for identification of appropriate

agents. For instance, the ability of a monoclonal antibody to inhibit adhesion of the natural cellular ligands to the cells expressing ICAM or the LFA-1 integrin can be used. Typically, the agent of the invention is incubated with the ICAM/LFA-1 bearing cells in the presence of the natural receptor/ligand-bearing cells, wherein the ICAM-bearing cells have been immobilized on a solid support. Inhibition of the cellular adhesion is then assessed by either calculating the amount of the bound mAb or assessing the displaced cells.

Agents effective in blocking or preventing the association of ICAM with lymphocytes may be identified by *in vivo* assays.

The specificity or discrimination between two or more competing substrates is determined by the ratios of bound to unbound. For example, according to a cellular assay such as those described herein, radiolabeled or flourescent labeled LFA-1 is incubated with immobilized ICAM receptor-immunoglobulin chimeras in varying concentration of unlabeled candidate compound. Increasing concentrations of successful candidate molecule effectively prevent binding of labeled LFA-1 to immobilized receptor chimeras. The concentration of unlabeled agent at which 50% maximal LFA-1 is displaced is referred to as the EC50 and reflects the receptor binding affinity. Therefore a candidate compound with an EC50 of 100 nM displays a substantially weaker interaction with a receptor than candidate agent with an EC50 of 10 nM. This discrimination in substrate specificity indicates that the preferred agent or antagonist has utility in, for example, preventing or blocking the interaction of ICAM with leukocyte surface antigens and especially LFA-1 in a setting where both the natural ligand and the so-called agent or antagonist are present.

An exemplary agent is a monoclonal antibody reactive with LFA-1 or ICAM. Antibodies are assessed by affinity constants. Affinity constants are a measure of the interaction between a particular ligand and its cognate receptor. The "binding affinity" or the measure of the strength of association between a particular receptor ligand interaction is generally measured by affinity constants for the equilibrium concentrations of associated and dissociated configurations of the ligand and its receptor. The present invention contemplates such an interaction between an agent or composition and the endothelial cell adhesion molecule ICAM. In general, the dissociation constants of ligand/integrin interactions in solution are relatively weak and range from low micromolar to high nanomolar. Additivity of multiple adhesive interactions at a cell surface, or the "avidity," provides the necessary binding energy to anchor leukocytes to the vascular endothelium. Therefore, in general, a useful composition or agent has a higher affinity for the integrin receptor than its native ligand. Such an antagonist blocks or prevents a high percentage of the cell surface interactions involved in cellular adhesion mediated by the LFA-1/ICAM interaction. Preferably the binding of the agent or antagonist should occur at an affinity of about $k_a = 10^{-4}$M or greater to be useful for the present invention, with greater than about 10^{-8}M being more preferable, and most preferably between about 10^{-8}M and about 10^{-10}M.

As additional criteria, those forms of the molecule that are readily absorbed by tissues, that are protected from rapid metabolism and/or that provide for prolonged half life, are preferentially selected in producing the compositions of the invention. One skilled in the art may also effect modifications of the protein formulation, to effect absorption. These modifications include, but are not limited to, use of a

pro-drug and chemical modification of the primary structure (Wearley, L.L., 1991, Crit. Rev. in Ther. Drug Carrier Systems, 8(4):333). In minimizing metabolism of the protein and thereby increasing the effective amount of protein, such modifications include but are not limited to chemical modifications and covalent attachment to a polymer (Wearley, L.L., 1991, *supra*).

Therapeutic Methods and Pharmaceutical Compositions

While not intending to be limited by a mechanism of action, it is believed that migration of activated leukocytes from the blood stream into the liver tissue is dependent on the interaction of the lymphocytes with the ICAM in the liver tissue. Leukocyte traffic across the vessel walls to extravascular tissue is necessary for host defense against microbial organisms or foreign antigens and repair of tissue damage. Under some circumstances, however, leukocyte-endothelial interactions may have deleterious consequences for the host. During the process of adherence and transendothelial migration, leukocytes may release products such as oxidants, proteases, or cytokines that directly damage endothelium or cause endothelial damage by releasing a variety of inflammatory mediators. The interaction of ICAM with leukocyte surface molecules, such as LFA-1, facilitates leukocyte migration and activation and contributes to the destructive effects of the inflammatory process.

Therefore, according to the present invention, agents that prevent the interaction between hepatically expressed ICAM and ligands such as LFA-1 can be employed to treat these types of disorders in the liver.

Additionally, the pharmaceutical compositions of the present invention can be used to eliminate or block the injury occurring in transplanted livers.

The preclinical and clinical therapeutic use of the present invention in the treatment of diseases or disorders associated with ICAM will be best accomplished by those of skill, employing accepted principles of diagnosis and treatment. Such principles are known in the art, and are set forth, for example, in Braunwald *et al.*, eds., Harrison's Principles or International Medicine, 11th Ed., McGraw-Hill, H.Y. (1987).

The most effective mode of administration and dosage regimen of agent will depend on the type of disease to be treated, the severity and course of the disease, whether the agents are administered for prophylactic or therapeutic purposes, previous therapy, the patient's clinical history and response to the agents such as antibodies, and the discretion of the attending physician. The agent is suitably administered to the patient at one time or over a series of treatments.

For most therapeutic applications, the agents may be administered to a mammal, preferably a patient, in a pharmaceutically acceptable dosage form, including those that may be administered to a patient intravenously as a bolus or by continuous infusion over a period of minutes, hours, days, weeks, or months, intramuscularly, subcutaneously, intra-articularly, intrasynovially, intrathecally, or periostally, or by oral, topical, or inhalation routes.

A dose of agent may be administered to the patient in one or more single administrations, continuous infusion, or bolus injection. For example, an initial dose of the agent is administered to the patient by injection or infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs.

However, other dosage regimens may be useful. According to another embodiment of the invention, the effectiveness of the agent may be improved by administering the agent serially or in combination with another agent that is effective for this purpose (for example, interferon).

The compositions of the present invention may be part of a delivery system such as liposomes. Delivery systems involving liposomes are discussed in International Patent Publication No. WO 91/02805 and International Patent Publication No. WO 91/19501, as well as U.S. Patent No. 4,880,635 to Janoff *et al.* These publications and patents provide useful descriptions of techniques for liposome drug delivery.

The compositions of the invention can be administered to a subject in need thereof to treat the subject by either prophylactically preventing a disease state or relieving it after it has begun. The pharmaceutical compositions of the invention may be administered in any suitable manner, including parental, topical, oral, or local (such as aerosol or transdermal) or any combination thereof. The compositions are preferably administered with a pharmaceutically acceptable carrier, the nature of the carrier differing with the mode of administration, for example, oral administration, usually using a solid carrier and I.V. administration a liquid salt solution carrier.

The compositions of the present invention include pharmaceutically acceptable components that are compatible with the patient and the protein and carbohydrate moieties of the compositions of the invention. These generally include suspensions, solutions and elixirs, and most especially biological buffers, such as phosphate buffered saline, saline, Dulbecco's Media, and the like. Aerosols may also be used, or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, and the like (in the case of oral solid preparations, such as powders, capsules, and tablets).

As used herein, the term "pharmaceutically acceptable" preferably means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals, and more particularly in humans.

The formulation of choice can be accomplished using a variety of the aforementioned buffers, or even excipients including, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin cellulose, magnesium carbonate, and the like. "Peglation" of the compositions may be achieved using techniques known to the art (see for example International Patent Publication No. WO92/16555, U.S. Patent No. 5,122,614 to Enzon, and International Patent Publication No. WO92/00748). Oral compositions may be taken in the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations, or powders.

A sufficient amount of the compositions of the invention should be administered to the patient to ensure that a substantial amount of the interaction between ICAM and a binding partner is inhibited. In this way, hepatic inflammation can either be prevented or ameliorated. The selection of compositions, frequency of administration, and amount of composition so administered will be in accordance with the particular disease being treated and its severity, the type of agent employed, the method of administration, the overall condition of the patient, and the judgment of the treating physician. Typical dosing regions will be analogous to treatment of these disease states by the use of antibodies and other biologicals. Typically, the compositions of the instant invention will contain from about 1% to about 95% of the

active ingredient, preferably about 10% to about 50%. Preferably, the dosing will be between about 0.25-100 mg/kg. About 1 mg to about 50 mg will be administered to a child, and between about 25 mg and about 1000 mg will be administered to an adult. An LFA-1 antagonist, humanized anti-CD11a antibody hu1124, can be administered at a dosage range of between 0.25 mg/kg to 10 mg/kg, preferably between 0.5-5.0 mg/kg, even more preferably between 0.7-2.0 mg/kg. A lower "conditioning dose" can be administered initially, followed by the therapeutically effective dose. Other effective dosages can be readily determined by one of the ordinary skill in the art through routine trials establishing dose response curves.

In determining the dosage of compositions to be administered, it must be kept in mind that one may not wish to completely block all of the ICAM molecules, or may wish to completely block such receptors for only a limited amount of time. In order for a normal healing process to proceed, at least some of the white blood cells or neutrophils must be brought into the tissue in the areas where the wound, infection or disease state is occurring. Thus, the dose of the composition administered as a blocking agent is adjusted based on the particular needs of the patient while taking into consideration a variety of factors such as the type of disease that is being treated.

Thus, an effective amount of a composition in accordance with the present invention is an amount effective to inhibit the interaction between ICAM and a ICAM-binding partner.

The following examples are offered by way of illustration and not by way of limitation. The disclosures of all citations in the specification are expressly incorporated herein by reference.

EXAMPLES

Example 1 - Generation of anti-CD11a antibodies

Humanized antibodies were prepared as described in WO 98/23761 and U.S. 6,037,454.

Example 2 - Treatment of Con-A induced Hepatitis in Mice

This animal model of Concanavalin-A induced hepatitis is well known in the art, see Tiegs G, et al. T cell-dependent experimental liver injury in mice inducible by concanavalin. A.J Clin Invest. 1992 Jul;90(1):196-203 and review article Tiegs G. Experimental hepatitis and role of cytokines. Acta Gastroenterol Belg. 1997 Apr-Jun;60(2):176-9.

Hepatitis was successfully induced in all animals treated with ConA. Hepatitis was scored using standard methods for grading hepatitis clinically. There was a significant reduction in the severity of the hepatitis, as determined by morphological criteria, organ weight / body weight and organ weight / brain weight ratios, in animals treated with anti-LFA-1/ CD11a. The severity of hepatitis was evaluated with a Histological Activity Index which was reduced in animals treated with the anti-CD11a antibodies.

Experimental Design:

Three groups of 6 animals were treated with ConA at 10mg/kg, in 200µl of PBS, on days 1, 2, 3, 6, 7 and 8. Group 3 served as an untreated control, i.e., no Con-A or antibody treatment. Group 1 was treated with a rodent isotype control antibody; group 2 with a rat anti-murine LFA-1 antibody, M17. Group 1 was treated with 300 ug antibody on days 0 and 5 only. Group 3 was treated with 300 ug on

alternate days. All animals were killed on day 9. Terminal bleeds were taken for liver function tests, organs were inspected and weighed at necropsy and the liver was processed for routine histology.

Results:

Con A induced a mild to moderate hepatitis, characterized by a lymphoblastic / lymphocytic infiltrate in portal tracts (portal triaditis), within the liver parenchyma proper (lobular hepatitis) and around terminal hepatic venules. The inflammatory infiltrate was accompanied in one animal by parenchymal necrosis. Hepatitis was scored using a modified version of the histological activity index (HAI), a semi-quantitative scoring system.

Treatment with anti-LFA1 significantly reduced hepatic inflammation (portal, lobular and perivenular) and liver injury. See Table 1 and Figures 1 and 2.

Table 1 Hepatitis scores:

Group Number	Group Label	Animal Alias	Portal Tract Inflammation (0-4)	Lobular Inflammation and Necrosis (0-4)	Lymphocytic Piecemeal Necrosis (0-4)	Hepatic Vein Inflammation (0-4)	Confluent Parenchymal Necrosis	Overall Grade (0-16)
1	Isotype	1	1	1	0	3	N	5
1	Isotype	2	3	1	1	3	N	8
1	Isotype	3	2	1	1	3	N	7
1	Isotype	4	2	1	1	2	N	6
1	Isotype	5	2	1	1	2	N	6
1	Isotype	6	1	1	1	2	N	5
1	Isotype	7	1	1	1	2	N	5
1	Isotype	8	2	2	1	3	N	8
1	Isotype	9	2	1	1	2	N	6
1	Isotype	10	2	3	1	2	Y	8
2	anti-CD11a	21	0	0	0	1	N	1
2	anti-CD11a	22	0	0	0	1	N	1
2	anti-CD11a	23	0	0	0	1	N	1
2	anti-CD11a	24	0	0	0	1	N	1
2	anti-CD11a	25	0	0	0	1	N	1
2	anti-CD11a	26	1	0	0	1	N	2
2	anti-CD11a	27	0	0	0	1	N	1
2	anti-CD11a	28	0	0	0	1	N	1
2	anti-CD11a	29	0	0	0	1	N	1
2	anti-CD11a	30	0	0	0	1	N	1
3	None	41	0	0	0	0	N	0
3	None	42	0	0	0	0	N	0
3	None	43	0	0	0	0	N	0
3	None	44	0	0	0	0	N	0
3	None	45	0	0	0	0	N	0

3	None	46	0	0	0	0	N	0
3	None	47	0	0	0	0	N	0
3	None	48	0	0	0	0	N	0
3	None	49	0	0	0	0	N	0
3	None	50	0	0	0	0	N	0

Figure 1 is a graph showing the effect of treatment on portal tract inflammation which is graded on a scale of 0-4. Figure 2 shows the overall grade or hepatitis score on a scale of 0-16. The cell mean on the y-axis indicates the grade of inflammation. The tables showing the statistical analysis below demonstrated the statistical significance of the results. These experiments were repeated and the results were reproducible with the same statistical significance.

Unpaired t-test for Portal Tract Inflammation (0-4)
Grouping Variable: Group Label
Hypothesized Difference = 0

		Mean Diff.	DF	t-Value	P-Value
anti-CD11a/LFA1	Isotype	-1.700	18	-7.603	<.0001
anti-CD11a/LFA1	None	.080	18	.793	.4381
Isotype	None	1.780	18	8.880	<.0001

Group Info for Portal Tract Inflammation (0-4)
Grouping Variable: Group Label

	Count	Mean	Variance	Std. Dev.	Std. Err.
anti-CD11a/LFA1	10	.100	.100	.316	.100
Isotype	10	1.800	.400	.632	.200
None	10	.020	.002	.042	.013

Unpaired t-test for Overall Grade (0-16)
Grouping Variable: Group Label
Hypothesized Difference = 0

		Mean Diff.	DF	t-Value	P-Value
anti-CD11a/LFA1	Isotype	-5.300	18	-12.854	<.0001
anti-CD11a/LFA1	None	1.000	18	10.000	<.0001
Isotype	None	6.300	18	15.750	<.0001

Group Info for Overall Grade (0-16)
Grouping Variable: Group Label

	Count	Mean	Variance	Std. Dev.	Std. Err.
anti-CD11a/LFA1	10	1.100	.100	.316	.100
Isotype	10	6.400	1.600	1.265	.400
None	10	.100	0.000	0.000	0.000

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the cultures deposited, since the deposited embodiments are intended as an illustration of an aspect of the invention and any cultures that are functionally equivalent are within the scope of this invention. The deposit of material

herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustration that it represents.

WHAT IS CLAIMED IS:

1. A method of treating a hepatic disorder in a host in need thereof comprising administering to the host a therapeutically effective amount of an agent which prevents the interaction of ICAM with a ligand therefor.

2. A method of treating a hepatic disorder in a host in need thereof comprising administering to the host a therapeutically effective amount of an agent which binds to LFA-1.

3. The method of claim 2, wherein the agent binds CD11a.

4. The method of any one of the preceding claims, wherein the hepatic disorder is characterized by inflammation.

5. The method of claim 4, wherein the inflammation comprises activation of residual hepatic leukocytes and/or hepatic infiltration of leukocytes.

6. The method of claim 5, wherein the agent prevents the interaction of ICAM with the infiltrating leukocytes.

7. The method of any one of the preceding claims, wherein the agent prevents the interaction of ICAM with CD11a expressing leukocytes.

8. The method of any one of the preceding claims, wherein the hepatic disorder is an infection, an iatrogenic disorder, a hereditary disorder, a cholestatic disorder, sarcoidosis, or liver injury resulting from graft versus host disease or graft rejection.

9. The method of claim 8, wherein the infection is a viral infection.

10. The method of claim 9, wherein the viral infection is a hepatitis infection.

11. The method of claim 10, wherein the hepatitis infection is a Hepatitis C infection.

12. The method of claim 8, wherein the cholestatic disorder is primary biliary cirrhosis, primary sclerosing cholangitis or adult idiopathic ductopenia.

13. The method of any one of the preceding claims, wherein the agent is an antibody.

14. The method of claim 13, wherein the antibody is a monoclonal antibody or an antigen binding fragment thereof.

15. The method of any one of the preceding claims, wherein the agent is an immunoadhesin comprising a ligand-binding portion of ICAM fused to an immunoglobulin constant domain.

16. The method of any one of the preceding claims, wherein the immunoadhesin comprises an LFA-1 portion fused to an immunoglobulin constant domain.

17. An article of manufacture, comprising:

(a) a container;

(b) a label on said container; and

(c) a composition comprising an active agent contained within said container; wherein the composition is effective for treating a hepatic disorder, the label on said container indicates that the composition can be used for treating a hepatic disorder, and the active agent in said composition comprises an agent which prevents the interaction of ICAM with a ligand therefor, and optionally

(d) a second container comprising a pharmaceutically-acceptable buffer; and

(e) instructions for using the composition to treat a hepatic disorder.

18. The article of claim 17, wherein the hepatic disorder is an infection, an iatrogenic disorder. a hereditary disorder, a disorder of unknown etiology, a cholestatic disorder, sarcoidosis, or liver injury in graft versus host disease or in graft rejection.

19. The article of claim 18, wherein the infection is a viral infection.

20. The article of claim 19, wherein the viral infection is a Hepatitis C infection.

21. A method of inhibiting the binding between a first cell expressing a ligand for ICAM and a second cell expressing ICAM, wherein the second cell is present in a liver, comprising contacting one or both of the cells with an agent which prevents the interaction of ICAM with the ICAM ligand.

22. The method of claim 21, wherein the contacting occurs *in vivo*.

23. The method of claim 21 wherein the second cell is a hepatocyte.

24. The method of claim 21 wherein the ligand for ICAM is LFA-1 and the agent is an anti-LFA-1 antibody.

25. The method of claim 24, wherein the antibody is an anti-CD11a antibody.

26. The method of claim 25, wherein the antibody is a humanized antibody.

27. A method of diagnosing a hepatic disorder comprising:

(a) contacting cells present in the liver with an agent that binds to ICAM; and

(b) detecting the binding thereof.

28. The method of claim 27, wherein the agent is a polyclonal antibody, a monoclonal antibody or an antigen-binding fragment thereof.

29. The method of claim 28, wherein the agent is detectably labeled.

30. The method of claim 28, further comprising the step of removing the cells from the organism in which they naturally occur prior to contacting with the agent.

31. A method for diagnosing hepatic disease or disorder in a subject, the method comprising the steps of:

(a) obtaining a serum sample from the subject;

(b) contacting the sample with at least one binding partner specific for a ICAM molecule under conditions which allow specific binding;

(c) measuring the amount of specific binding that occurs between a component in the sample and at least one binding partner, wherein the amount of specific binding indicates the amount of ICAM expressed in the sample; and

(d) comparing the amount of specific binding measured in step (c) with a baseline amount of specific binding,

wherein an increase in the amount of specific binding in the sample indicates the presence of the hepatic disease or disorder.

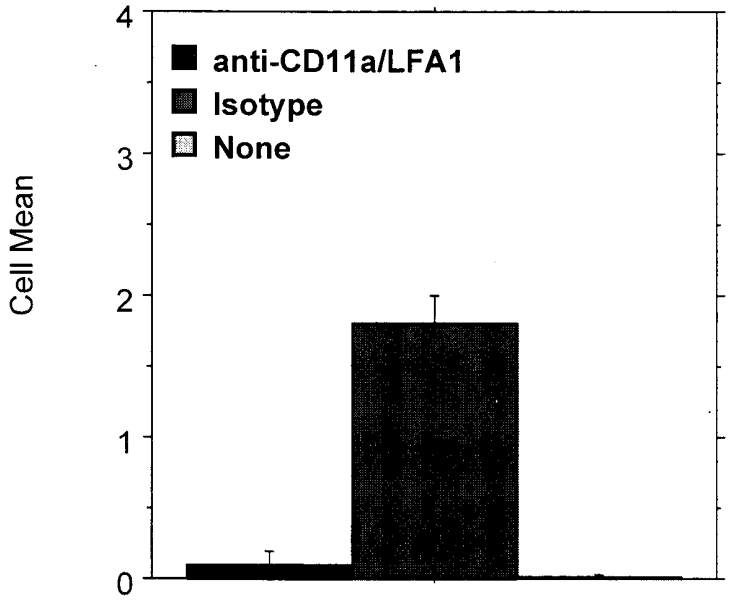


Figure 1

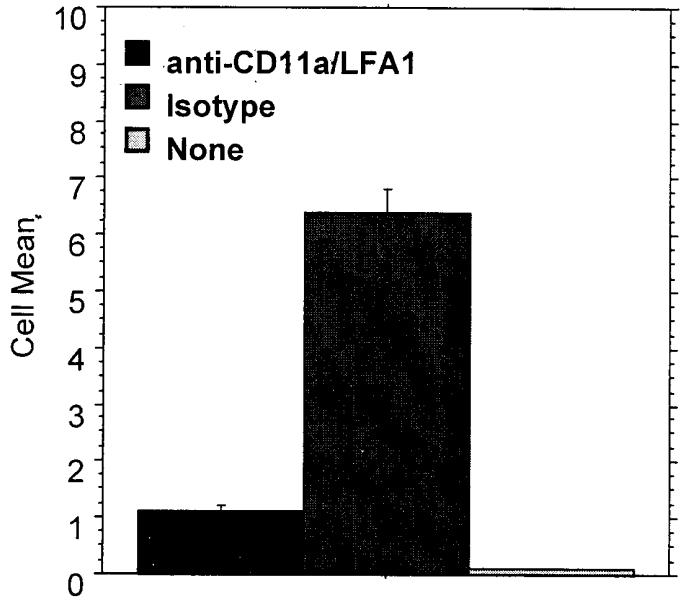


Figure 2

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Internal Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/395 G01N33/577 A61P1/16 A61P37/06 A61K38/17
//C07K16/28,C07K19/00,C07K14/705

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)
IPC 7 C07K

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 practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, EMBASE, CHEM ABS Data, LIFESCIENCES

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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

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"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 when the document is taken alone

"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

Date of the actual completion of the international search	Date of mailing of the international search report
18 May 2001	01/06/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Wagner, R

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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