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(54) Title: TREATMENT OF LIVER DISEASES WITH POLYAMINE SYNTHESIS INHIBITORS

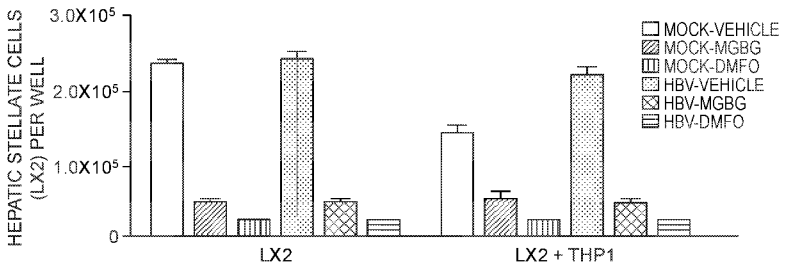

FIG. 2A

(57) Abstract: The present invention relates to methods of inhibiting activation of M2-like macrophages in a subject, inhibiting liver inflammation and/or fibrosis, and treating liver diseases in a subject using polyamine synthesis inhibitors.

Treatment of Liver Diseases with Polyamine Synthesis Inhibitors

RELATED APPLICATIONS

[0001] The present application claims the benefit, under 35 U.S.C. § 119(e), of U.S. Provisional Application No. 62/162,310, filed May 15, 2015, the content of which is incorporated by reference herein in its entirety.

STATEMENT OF FEDERAL SUPPORT

[0002] This invention was made with government support under Grant Nos. AI050410, DK095962, P30 AI50410, and AI095097 awarded by the National Institutes of Health. The government has certain rights in this invention.

FIELD OF THE INVENTION

[0003] The present invention relates to methods of inhibiting activation of M2-like macrophages in a subject, inhibiting liver inflammation and/or fibrosis, and treating liver diseases in a subject using polyamine synthesis inhibitors.

BACKGROUND OF THE INVENTION

[0004] Chronic hepatitis B virus (HBV) infection is a major global health burden, resulting in liver cirrhosis and development of hepatocellular carcinoma (HCC) (Zhang *et al., Blood 118*:1912 (2011); Choi *et al., J. Clin. Gastroenterol. 46*:413 (2012)). Chronic HBV infection is associated with impaired anti-viral immune responses and chronic inflammation in the liver, leading to progressive liver diseases including HCC. The development of preventive vaccines and therapeutics using chimpanzees and surrogate hepatitis virus-small animal models has played a significant role in preventing new infections and controlling HBV-induced liver diseases. However, HBV is endemic in many developing countries with over 350 million people worldwide chronically infected (Dandri *et al., Semin. Immunopathol. 35*:7 (2013)). Delineation of the mechanisms by which HBV evades host immunity to establish chronic infection and promote liver disease is hampered by the lack of robust animal models (Gilgenkrantz, *Med. Sci. (Paris) 27*:587 (2011); de Jong *et al., J. Clin. Invest. 120*:650 (2010); Brezillon *et al., PLoS One 6*: e25096 (2011)).

[0005] HBV and other human hepatotropic pathogens including hepatitis C virus (HCV) have host species restriction, namely humans and chimpanzees. Furthermore, chronic infection and associated immunopathogenesis including liver fibrosis is restricted to humans, as chimpanzees only develop transient acute infection with no chronic liver disease. To overcome host species restriction barrier for *in vivo* infection and disease modeling, we have recently developed a humanized mouse model with human immune system and liver cells (Washburn *et al., Gastroenterology 140*:1334 (2011); Robinet *et al., J. Hepatol. 55*:718 (2011)). The liver and immune system-humanized mouse model supports HCV/HBV infection in the liver and generates human T-cell response to HCV/HBV. Additionally, HCV/HBV infection induces liver inflammation and fibrosis, correlated with activation of human hepatic stellate cells and expression of human fibrogenic genes (Washburn *et al., Gastroenterology 140*:1334 (2011)). Additionally, we observed accumulation of activated human M2–like macrophages in the HBV-infected humanized liver. Importantly, similar M2-like macrophage accumulation was confirmed in chronic HBV patients and HBV-induced acute liver failure patients. Importantly, inoculation of human macrophages culture with HBV positive supernatant resulted in M2–like activation.

[0006] The present invention addresses previous shortcomings in the art by providing methods for treating liver diseases.

SUMMARY OF THE INVENTION

[0007] The present invention is based on the identification of the role of M2-like macrophage activation in liver inflammation and fibrosis, the inhibition of M2-like macrophage activation using polyamine synthesis inhibitors, and the effectiveness of the same in treating liver diseases.

[0008] Accordingly, in one aspect, the invention relates to a method of inhibiting activation of M2-like macrophages in a subject, comprising delivering to the subject an effective amount of a polyamine synthesis inhibitor, thereby inhibiting activation of M2-like macrophages in the subject.

[0009] A further aspect of the invention relates to a method of decreasing the number of M2-like macrophages in a subject, comprising delivering to the subject an

effective amount of a polyamine synthesis inhibitor, thereby decreasing the level of M2-like macrophages in the subject.

[0010] Another aspect of the invention relates to a method of inhibiting liver inflammation and/or fibrosis in a subject in need thereof, comprising inhibiting the activation of M2-like macrophages in the subject, thereby inhibiting liver inflammation and/or fibrosis in the subject.

[0011] An additional aspect of the invention relates to a method of inhibiting liver inflammation and/or fibrosis in a subject in need thereof, comprising decreasing the number of M2-like macrophages in the subject, thereby inhibiting liver inflammation and/or fibrosis in the subject.

[0012] A further aspect of the invention relates to a method of treating liver disease in a subject in need thereof, comprising inhibiting the activation of M2-like macrophages in the subject, thereby treating liver disease in the subject.

[0013] Another aspect of the invention relates to a method of treating liver disease in a subject in need thereof, comprising decreasing the number of M2-like macrophages in the subject, thereby treating liver disease in the subject.

[0014] These and other aspects of the invention are set forth in more detail in the description of the invention below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] Figures 1A-1C show that polyamine synthesis inhibitors attenuate HBV-induced M2 macrophage activation to promote anti-viral M1 response. (**A**) Human monocytic cell line (THP1) was inoculated with cell culture derived HBV for 6 days in the presence or absence of MGBG (1μM) and macrophage polarization was examined. M1 (iNOS) and M2 (ARG1) macrophage activation markers were examined using gene expression analysis. (**B-C**) Human primary M1 or M2 polarized macrophages were inoculated with cell culture derived HBV for 6 days in the presence or absence of MGBG (1μM) (**B**) or DFMO (1mM) (**C**) and macrophage activation/polarization was examined. M1 (IL10) and M2 (IL12) macrophage activation markers were examined using cytokine analysis.

[0016] Figures 2A-2B show that polyamine synthesis inhibitors attenuate HBV-activated monocyte/macrophage induced hepatic stellate cell activation. (**A-B**) Human hepatic stellate cell line (LX2) was cultured in the absence or presence of

HBV and in the absence or presence of human monocytic cell line (THP1) with or without polyamine synthesis inhibitor treatment; hepatic stellate cell expansion was examined.

[0017] Figure 3 shows that polyamine synthesis inhibitors attenuate HBV-induced M2-like macrophages in humanized mice. A2/NSG/Fas-hu mice or non-humanized mice were inoculated with PBS or HBV (10^7 GE/mouse) and HBV infected animals were randomly assigned to vehicle or polyamine synthesis inhibitor treatment group at 8 weeks post infection and treated with MGBG for 5 weeks (1X per week). M2-like macrophages ($hCD68^+$ $hCD163^+$ $hiNOS^-$) were examined at 16 weeks post infection using immunohistochemistry.

[0018] Figure 4 shows that polyamine synthesis inhibitors attenuate chronic HBV infection and associated liver disease. A2/NSG/Fas-hu mice or non-humanized mice were inoculated with PBS or HBV (10^7 GE/mouse) and HBV infected animals were randomly assigned to vehicle or polyamine synthesis inhibitor treatment group at 8 weeks post infection and treated with MGBG for 5 weeks (1X per week). HBV infection (HBsAg immunohistochemistry), liver inflammation (H/E), cirrhosis (Sirius Red/Fast Green) were examined at 16 weeks post infection.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The present invention will now be described in more detail with reference to the accompanying drawings, in which preferred embodiments of the invention are shown. This invention may, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

[0020] Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a complex comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

[0021] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention.

[0022] All publications, patent applications, patents, patent publications and other references cited herein are incorporated by reference in their entireties for the teachings relevant to the sentence and/or paragraph in which the reference is presented.

[0023] As used in the description of the invention and the appended claims, the singular forms "a," "an," and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0024] Also as used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

[0025] The term "about," as used herein when referring to a measurable value such as an amount of polypeptide, dose, time, temperature, enzymatic activity or other biological activity and the like, is meant to encompass variations of ± 20%, ± 10%, ± 5%, ± 1%, ± 0.5%, or even ± 0.1% of the specified amount.

[0026] The transitional phrase "consisting essentially of" means that the scope of a claim is to be interpreted to encompass the specified materials or steps recited in the claim, "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention. *See*, *In re Herz*, 537 F.2d 549, 551-52, 190 USPQ 461, 463 (CCPA 1976) (emphasis in the original); *see also* MPEP § 2111.03.

[0027] The term "inhibit" or "reduce" or grammatical variations thereof as used herein refers to a decrease or diminishment in the specified level or activity of at least about 15%, 25%, 35%, 40%, 50%, 60%, 75%, 80%, 90%, 95% or more. In particular embodiments, the inhibition or reduction results in little or essentially no detectible activity (at most, an insignificant amount, *e.g.*, less than about 10% or even 5%).

[0028] An "effective" amount as used herein is an amount that provides a desired effect.

[0029] A "therapeutically effective" amount as used herein is an amount that provides some improvement or benefit to the subject. Alternatively stated, a

"therapeutically effective" amount is an amount that will provide some alleviation, mitigation, or decrease in at least one clinical symptom in the subject. Those skilled in the art will appreciate that the therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject.

[0030] By the terms "treat," "treating," or "treatment," it is intended that the severity of the subject's condition is reduced or at least partially improved or modified and that some alleviation, mitigation or decrease in at least one clinical symptom is achieved.

[0031] The terms "prevent," "preventing," and "prevention" (and grammatical variations thereof) refer to a decrease and/or delay in the extent or severity of a disease, disorder and/or clinical symptom(s) after onset relative to what would occur in the absence of carrying out the methods of the invention prior to the onset of the disease, disorder and/or clinical symptom(s).

[0032] "Concurrently" means sufficiently close in time to produce a combined effect (that is, concurrently can be simultaneously, or it can be two or more events occurring within a short time period before or after each other). In some embodiments, the administration of two or more compounds "concurrently" means that the two compounds are administered closely enough in time that the presence of one alters the biological effects of the other. The two compounds can be administered in the same or different formulations or sequentially. Concurrent administration can be carried out by mixing the compounds prior to administration, or by administering the compounds in two different formulations, for example, at the same point in time but at different anatomic sites or using different routes of administration.

[0033] One aspect of the invention relates to a method of inhibiting activation of M2-like macrophages in a subject, comprising delivering to the subject an effective amount of a polyamine synthesis inhibitor, thereby inhibiting activation of M2-like macrophages in the subject. An "M2-like macrophage," as used herein, refers to a macrophage with the prototypical markers $CD68^+$ $CD163^+$ $iNOS^-$ that synthesizes polyamines during activation and exhibits pro-fibrogenic, pro-tumorigenic/carcinogenic and immunosuppressive properties. In some embodiments, activation of M2-like macrophages is inhibited by at least about 15%, *e.g.*, at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more.

[0034] Another aspect of the invention relates to a method of decreasing the number of M2-like macrophages in a subject, comprising delivering to the subject an effective amount of a polyamine synthesis inhibitor, thereby decreasing the number of M2-like macrophages in the subject. In some embodiments, the number of M2-like macrophages is decreased by at least about 15%, *e.g.*, at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more. In some embodiments, the decrease in the number of M2-like macrophages is a decrease in the total number of M2-like macrophages. In other embodiments, the decrease in the number of M2-like macrophages is a decrease in the number of M2-like macrophages in a specific organ or region of the subject, *e.g.*, the liver or the blood stream.

[0035] A further aspect of the invention relates to a method of inhibiting liver inflammation and/or fibrosis in a subject in need thereof, comprising inhibiting the activation of M2-like macrophages in the subject, thereby inhibiting liver inflammation and/or fibrosis in the subject.

[0036] An additional aspect of the invention relates to a method of inhibiting liver inflammation and/or fibrosis in a subject in need thereof, comprising decreasing the number of M2-like macrophages in the subject, thereby inhibiting liver inflammation and/or fibrosis in the subject.

[0037] The phrase "inhibiting liver inflammation and/or fibrosis," as used herein encompasses a decrease in the amount of inflammation and/or fibrosis that was present prior to the method being carried out, a slowing of the rate of increase of inflammation and/or fibrosis that was present prior to the method being carried out, and/or a delay in the onset of inflammation and/or fibrosis.

[0038] Another aspect of the invention relates to a method of treating liver disease in a subject in need thereof, comprising inhibiting the activation of M2-like macrophages in the subject, thereby treating liver disease in the subject.

[0039] A further aspect of the invention relates to a method of treating liver disease in a subject in need thereof, comprising decreasing the number of M2-like macrophages in the subject, thereby treating liver disease in the subject.

[0040] An additional aspect of the invention relates to polyamine synthesis inhibitors for use in a method of inhibiting activation of M2-like macrophages in a subject, a method of decreasing the number of M2-like macrophages in a subject, a

method of inhibiting liver inflammation and/or fibrosis in a subject in need thereof, or a method of treating liver disease in a subject in need thereof.

[0041] Another aspect of the invention relates to polyamine synthesis inhibitors for use in the manufacture of a medicament for inhibiting activation of M2-like macrophages in a subject, decreasing the number of M2-like macrophages in a subject, inhibiting liver inflammation and/or fibrosis in a subject in need thereof, or treating liver disease in a subject in need thereof.

[0042] The phrase "treating liver disease," as used herein encompasses alleviating, mitigating, or decreasing at least one clinical symptom of the liver disease, slowing of progression of the liver disease, and delaying or minimizing the risk of progression of a less serious disease to a more serious disease, *e.g.*, inflammation to fibrosis, fibrosis to cirrhosis, or cirrhosis to HCC.

[0043] The liver disease can be any known liver disease, including without limitation HCC, cirrhosis, fibrosis, organ transplant rejection, veno-occlusive disease, sinusoidal obstruction syndrome, hepatitis virus infection (*e.g.*, A, B, C, D, E, or G), non-alcoholic fatty liver disease, alcoholic liver disease, alcohol- or drug-induced hepatitis, steatohepatitis, autoimmune hepatitis, haemochromatosis, cholangiocarcinoma, metastatic cancers, Wilson's disease, Crigler-Najjar syndrome, primary sclerosing cholangitis, primary biliary cirrhosis, Budd-Chiari syndrome, protoporphyria, Gilbert's syndrome, rotor syndrome, glycogen storage disease type 2, hemangioma, hyperbilirubinemia, biliary atresia, Byler disease, Dubin-Johnson syndrome, alpha-1 antitrypsin deficiency, Caroli disease, Alagille syndrome, and progressive familial intrahepatic cholestasis, or any combination thereof. In some embodiments, the liver disease is an inflammatory disease, *e.g.*, a chronic inflammatory disease. In some embodiments, the liver disease is fibrosis, cirrhosis, hepatitis infection, and/or HCC.

[0044] In certain embodiments, the methods of inhibiting liver inflammation and/or fibrosis in a subject or treating liver disease in a subject comprise delivering to the subject a therapeutically effective amount of a polyamine synthesis inhibitor.

[0045] The polyamine synthesis pathway is well-known in the art. The enzymes involved in the pathway include ornithine decarboxylase (ODC), S-adenosylmethionine decarboxylase (SAMDC), arginase, spermidine synthase, and spermine synthase. In certain embodiments, the polyamine synthesis inhibitor is an

inhibitor of an enzyme in the polyamine synthesis pathway. In some embodiments, the polyamine synthesis inhibitor is a compound that inhibits a polyamine synthesis enzyme by at least 50%, *e.g.*, by at least 50%, 60%, 70%, 80%, 90%, or more. Inhibitors of enzymes in the polyamine synthesis pathway are also well known and include, without limitation, α-difluoromethylornithine, α-methylornithine, monofluoromethyldehydroornithine methylester, α-difluoromethylarginine, methylglyoxal bis(guanylhydrazone), (N^1,N^{12}-bis-(ethyl)-spermine, 1,19-bis-(ethylamino)-5,10,15, triazononadecane, and sardomozide. In some embodiments, the inhibitor is an inhibitor of ODC or SAMDC.

[0046] In some embodiments, the methods of the invention further comprise delivering to the subject a liver disease therapeutic agent in addition to the polyamine synthesis inhibitor or providing other types of treatment for liver disease. Examples include, without limitation, surgery, radiation, chemotherapy, antiviral therapy, liver transplantation, immunostimulation, change in diet, avoidance of alcohol and/or drugs, and supportive care. Numerous liver disease therapeutic agents are known in the art, and include, without limitation, interferon, pegylated interferon, lamivudine, adefovir, entecavir, tenofovir, telbivudine, ribavirin, sofosbuvir, simeprevir, imeprevir, sorafenib, ^{131}I-lipiodol, percutaneous ethanol injection, cisplatin, doxorubicin, epirubicin, fluorouracil, taxol, leucovorin, gemcitabine, irinotecan, capecitabine, erlotinib, antibiotics, ursodeoxycholic acid, ursodiol, immunosuppressants (*e.g.*, corticosteroids, cyclosporin, tacrolimus, azathioprine, mycophenolic acid, sirolimus, everolimus, basiliximab, daclizumab), defibrotide, deferoxamine, deferasirox, deferiprone, penicillamine, trientine, and tetrathiomolybdate.

[0047] The present invention finds use in veterinary and medical applications as well as research applications. As used herein, the term "subject" refers to humans and other animals. Suitable subjects include mammals such as humans, as well as those mammals of importance due to being endangered, such as Siberian tigers; of economic importance, such as animals raised on farms; animals of social importance to humans, such as animals kept as pets or in zoos; and research animals, such as mice, rabbits, guinea pigs, ferrets, dogs, cats, monkeys, and apes. Examples of such animals include but are not limited to: carnivores such as cats and dogs; swine, including pigs, hogs, and wild boars; ruminants and/or ungulates such as cattle, oxen,

sheep, giraffes, deer, goats, bison, and camels; horses; and poultry. Human subjects include neonates, infants, juveniles, and adults. In some embodiments, the subject is an animal model of liver disease.

[0048] In some embodiments, the subject is one that has a liver disease, has had a liver disease, or is at risk for a liver disease. A subject at risk for a liver disease, *e.g.*, HCC or cirrhosis, can be one that has a family history of the disease, is genetically predisposed to the disease, or has symptoms, habits, or other diseases known to lead to the disease. In one embodiment, the subject has liver fibrosis. In another embodiment, the subject has liver cirrhosis. In a further embodiment, the subject has dysplastic lesions and/or regenerative nodules in the liver. In another embodiment, the subject has hepatitis A, B, or C. In a further embodiment, the subject is an alcoholic or a drug user (*e.g.*, a recreational drug user or drug addict). In some embodiments, the subject is obese. In other embodiments, the subject has diabetes and/or another metabolic disorder.

[0049] As a further aspect, the invention provides pharmaceutical formulations and methods of administering the same to treat liver disease. The pharmaceutical formulation may comprise any of the reagents discussed above in a pharmaceutically acceptable carrier.

[0050] By "pharmaceutically acceptable" it is meant a material that is not biologically or otherwise undesirable, *i.e.*, the material can be administered to a subject without causing any undesirable biological effects such as toxicity.

[0051] The formulations of the invention can optionally comprise medicinal agents, pharmaceutical agents, carriers, adjuvants, dispersing agents, diluents, and the like.

[0052] The compounds of the invention can be formulated for administration in a pharmaceutical carrier in accordance with known techniques. *See, e.g.,* Remington, *The Science And Practice of Pharmacy* (21th Ed. 2005). In the manufacture of a pharmaceutical formulation according to the invention, the compound (including the physiologically acceptable salts thereof) is typically admixed with, *inter alia*, an acceptable carrier. The carrier can be a solid or a liquid, or both, and is preferably formulated with the compound as a unit-dose formulation, for example, a tablet, which can contain from 0.01 or 0.5% to 95% or 99% by weight of the compound.

One or more compounds can be incorporated in the formulations of the invention, which can be prepared by any of the well-known techniques of pharmacy.

[0053] A further aspect of the invention is a method of treating subjects *in vivo*, comprising administering to a subject a pharmaceutical composition comprising a compound of the invention in a pharmaceutically acceptable carrier, wherein the pharmaceutical composition is administered in a therapeutically effective amount. Administration of the compounds of the present invention to a human subject or an animal in need thereof can be by any means known in the art for administering compounds.

[0054] The formulations of the invention include those suitable for oral, rectal, topical, buccal (*e.g.*, sub-lingual), vaginal, parenteral (*e.g.*, subcutaneous, intramuscular including skeletal muscle, cardiac muscle, diaphragm muscle and smooth muscle, intradermal, intravenous, intraperitoneal), topical (*i.e.*, both skin and mucosal surfaces, including airway surfaces), intranasal, transdermal, intraarticular, intrathecal, and inhalation administration, administration to the liver by intraportal delivery, as well as direct organ injection (*e.g.*, into the liver, into the brain for delivery to the central nervous system, into the pancreas, or into a tumor or the tissue surrounding a tumor). In some embodiments, the formulation is delivered to the site of tissue damage (*e.g.*, fibrosis) or inflammation. The most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular compound which is being used.

[0055] In certain embodiments, the inhibitor is administered via one or more of oral administration, injection, and a surgically implanted pump. In some embodiments, the administration is via intravenous injection, intraportal delivery, or direct liver injection.

[0056] For injection, the carrier will typically be a liquid, such as sterile pyrogen-free water, pyrogen-free phosphate-buffered saline solution, bacteriostatic water, or Cremophor EL[R] (BASF, Parsippany, N.J.). For other methods of administration, the carrier can be either solid or liquid.

[0057] For oral administration, the compound can be administered in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. Compounds can be encapsulated in gelatin capsules together with inactive ingredients and powdered carriers, such as glucose, lactose,

sucrose, mannitol, starch, cellulose or cellulose derivatives, magnesium stearate, stearic acid, sodium saccharin, talcum, magnesium carbonate and the like. Examples of additional inactive ingredients that can be added to provide desirable color, taste, stability, buffering capacity, dispersion or other known desirable features are red iron oxide, silica gel, sodium lauryl sulfate, titanium dioxide, edible white ink and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric-coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

[0058] Formulations suitable for buccal (sub-lingual) administration include lozenges comprising the compound in a flavored base, usually sucrose and acacia or tragacanth; and pastilles comprising the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

[0059] Formulations of the present invention suitable for parenteral administration comprise sterile aqueous and non-aqueous injection solutions of the compound, which preparations are preferably isotonic with the blood of the intended recipient. These preparations can contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient. Aqueous and non-aqueous sterile suspensions can include suspending agents and thickening agents. The formulations can be presented in unit\dose or multi-dose containers, for example sealed ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use.

[0060] Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules and tablets of the kind previously described. For example, in one aspect of the present invention, there is provided an injectable, stable, sterile composition comprising a compound of the invention, in a unit dosage form in a sealed container. The compound or salt is provided in the form of a lyophilizate which is capable of being reconstituted with a suitable pharmaceutically acceptable carrier to form a liquid composition suitable for injection thereof into a subject. The

unit dosage form typically comprises from about 10 mg to about 10 grams of the compound or salt. When the compound or salt is substantially water-insoluble, a sufficient amount of emulsifying agent which is pharmaceutically acceptable can be employed in sufficient quantity to emulsify the compound or salt in an aqueous carrier. One such useful emulsifying agent is phosphatidyl choline.

[0061] Formulations suitable for rectal administration are preferably presented as unit dose suppositories. These can be prepared by admixing the compound with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

[0062] Formulations suitable for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which can be used include petroleum jelly, lanoline, polyethylene glycols, alcohols, transdermal enhancers, and combinations of two or more thereof.

[0063] Formulations suitable for transdermal administration can be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Formulations suitable for transdermal administration can also be delivered by iontophoresis (*see*, for example, Tyle, *Pharm. Res. 3*:318 (1986)) and typically take the form of an optionally buffered aqueous solution of the compound. Suitable formulations comprise citrate or bis\tris buffer (pH 6) or ethanol/water and contain from 0.1 to 0.2M of the compound.

[0064] The compound can alternatively be formulated for nasal administration or otherwise administered to the lungs of a subject by any suitable means, *e.g.*, administered by an aerosol suspension of respirable particles comprising the compound, which the subject inhales. The respirable particles can be liquid or solid. The term "aerosol" includes any gas-borne suspended phase, which is capable of being inhaled into the bronchioles or nasal passages. Specifically, aerosol includes a gas-borne suspension of droplets, as can be produced in a metered dose inhaler or nebulizer, or in a mist sprayer. Aerosol also includes a dry powder composition suspended in air or other carrier gas, which can be delivered by insufflation from an inhaler device, for example. *See* Ganderton & Jones, *Drug Delivery to the Respiratory Tract*, Ellis Horwood (1987); Gonda (1990) *Critical Reviews in Therapeutic Drug Carrier Systems* 6:273-313; and Raeburn *et al., J. Pharmacol. Toxicol. Meth.* 27:143 (1992). Aerosols of liquid particles comprising the compound

can be produced by any suitable means, such as with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer, as is known to those of skill in the art. *See, e.g.*, U.S. Patent No. 4,501,729. Aerosols of solid particles comprising the compound can likewise be produced with any solid particulate medicament aerosol generator, by techniques known in the pharmaceutical art.

[0065] Alternatively, one can administer the compound in a local rather than systemic manner, for example, in a depot or sustained-release formulation.

[0066] Further, the present invention provides liposomal formulations of the compounds disclosed herein. The technology for forming liposomal suspensions is well known in the art. When the compound is in the form of an aqueous-soluble material, using conventional liposome technology, the same can be incorporated into lipid vesicles. In such an instance, due to the water solubility of the compound, the compound will be substantially entrained within the hydrophilic center or core of the liposomes. The lipid layer employed can be of any conventional composition and can either contain cholesterol or can be cholesterol-free. When the compound of interest is water-insoluble, again employing conventional liposome formation technology, the compound can be substantially entrained within the hydrophobic lipid bilayer which forms the structure of the liposome. In either instance, the liposomes which are produced can be reduced in size, as through the use of standard sonication and homogenization techniques. The liposomal formulations containing the compound disclosed herein, can be lyophilized to produce a lyophilizate which can be reconstituted with a pharmaceutically acceptable carrier, such as water, to regenerate a liposomal suspension.

[0067] In the case of water-insoluble compounds, a pharmaceutical composition can be prepared containing the water-insoluble compound, such as for example, in an aqueous base emulsion. In such an instance, the composition will contain a sufficient amount of pharmaceutically acceptable emulsifying agent to emulsify the desired amount of the compound. Particularly useful emulsifying agents include phosphatidyl cholines and lecithin.

[0068] In addition to compound, the pharmaceutical compositions can contain other additives, such as pH-adjusting additives. In particular, useful pH-adjusting agents include acids, such as hydrochloric acid, bases or buffers, such as sodium lactate, sodium acetate, sodium phosphate, sodium citrate, sodium borate, or sodium

gluconate. Further, the compositions can contain microbial preservatives. Useful microbial preservatives include methylparaben, propylparaben, and benzyl alcohol. The microbial preservative is typically employed when the formulation is placed in a vial designed for multidose use. Other additives that are well known in the art include, *e.g.*, detackifiers, anti-foaming agents, antioxidants (*e.g.*, ascorbyl palmitate, butyl hydroxy anisole (BHA), butyl hydroxy toluene (BHT) and tocopherols, *e.g.*, α-tocopherol (vitamin E)), preservatives, chelating agents (*e.g.*, EDTA and/or EGTA), viscomodulators, tonicifiers (*e.g.*, a sugar such as sucrose, lactose, and/or mannitol), flavorants, colorants, odorants, opacifiers, suspending agents, binders, fillers, plasticizers, lubricants, and mixtures thereof. The amounts of such additives can be readily determined by one skilled in the art, according to the particular properties desired.

[0069] The additive can also comprise a thickening agent. Suitable thickening agents can be those known and employed in the art, including, *e.g.*, pharmaceutically acceptable polymeric materials and inorganic thickening agents. Exemplary thickening agents for use in the present pharmaceutical compositions include polyacrylate and polyacrylate co-polymer resins, for example poly-acrylic acid and poly-acrylic acid/methacrylic acid resins; celluloses and cellulose derivatives including: alkyl celluloses, *e.g.*, methyl-, ethyl- and propyl-celluloses; hydroxyalkyl-celluloses, *e.g.*, hydroxypropyl-celluloses and hydroxypropylalkyl-celluloses such as hydroxypropyl-methyl-celluloses; acylated celluloses, *e.g.*, cellulose-acetates, cellulose-acetatephthallates, cellulose-acetatesuccinates and hydroxypropylmethyl-cellulose phthallates; and salts thereof such as sodium-carboxymethyl-celluloses; polyvinylpyrrolidones, including for example poly-N-vinylpyrrolidones and vinylpyrrolidone co-polymers such as vinylpyrrolidone-vinylacetate co-polymers; polyvinyl resins, *e.g.*, including polyvinylacetates and alcohols, as well as other polymeric materials including gum traganth, gum arabicum, alginates, *e.g.*, alginic acid, and salts thereof, *e.g.*, sodium alginates; and inorganic thickening agents such as atapulgite, bentonite and silicates including hydrophilic silicon dioxide products, *e.g.*, alkylated (for example methylated) silica gels, in particular colloidal silicon dioxide products. Such thickening agents as described above can be included, *e.g.*, to provide a sustained release effect. However, where oral administration is intended, the use of thickening agents as aforesaid will generally not be required and is generally less

preferred. Use of thickening agents is, on the other hand, indicated, *e.g.*, where topical application is foreseen.

[0070] In particular embodiments, the compound is administered to the subject in a therapeutically effective amount, as that term is defined above. Dosages of pharmaceutically active compounds can be determined by methods known in the art, *see, e.g.,* Remington, *The Science And Practice of Pharmacy* (21th Ed. 2005). The therapeutically effective dosage of any specific compound will vary somewhat from compound to compound, and patient to patient, and will depend upon the condition of the patient and the route of delivery. In one embodiment, the compound is administered at a dose of about 0.001 to about 10 mg/kg body weight, *e.g.*, about 0.001, 0.005, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mg/kg. In some instances, the dose can be even lower, *e.g.*, as low as 0.0005 or 0.0001 mg/kg or lower. In some instances, the dose can be even higher, *e.g.*, as high as 20, 50, 100, 500, or 1000 mg/kg or higher. The present invention encompasses every sub-range within the cited ranges and amounts.

[0071] The present invention is more particularly described in the following examples that are intended as illustrative only since numerous modifications and variations therein will be apparent to those skilled in the art.

EXAMPLE 1

Experimental Procedures

[0072] **Isolation of $CD34^+$ HSC and liver progenitor cells from human fetal liver**: Human liver progenitor cells containing hepatoblasts (Hep) and $CD34^+$ hematopoietic stem cells (HSC) were isolated from 15-19 weeks old human fetal liver tissue (Advanced Bioscience Resources) essentially as described (Jiang *et al., Blood 112*:2858 (2008); Schmelzer *et al., Stem Cells 24*:1852 (2006); Zhang *et al., Blood 109*:2978 (2007)). To separate progenitor liver cells from non-parenchymal cells (including HSC), the fetal liver cells were centrifuged at low speed three times (5 minutes, 18xg). Liver progenitor cells were collected in the pellet. The supernatant was centrifuged at 469xg for 5 min to collect the non-parenchyma mononuclear cells. $CD34^+$ cells were isolated by magnetic-activated cell sorting (MACS), and the purity of $CD34^+$ HSCs was greater than 95%. Cell viability, measured using Guava Easycyte-with Viacount staining (Millipore), generally exceeded 90%.

[0073] Construction of A2/NSG/Fas-hu mice with human immune and liver cells: $CD34^+$ HSCs (0.5 - $1x10^6$) and Hep (liver) progenitor cells (0.5 - $1x10^6$) from the same donor liver were co-injected into the liver of 1 to 2 day old newborn A2/NSG mice, previously irradiated at 200 rad. Additionally, fetal thymus tissue from the same donor was also transplanted when available. Animals were injected 3-5 times via ip with Jo2 antibody/PBS at 0.1-0.15 mg/kg body weight (BD Pharmingen) every 4-5 days at approximately 3-4 weeks post transplant of human cells (Pajvani *et al., Nat. Med. 11*:797 (2005)). At 12-16 weeks post-transplant with HSC^+Hep cells, transplanted mice were bled to determine human leukocyte ($hCD45^+$) reconstitution by FACS and human albumin concentration in the blood by ELISA (Bethyl laboratories). All experiments using live rodents conformed to governmental and institutional guidelines.

[0074] HBV infection of humanized mice: HBV clinical isolates were obtained from patients with chronic HBV infection (SeraCare). A2/NSG-humanized mice or non-humanized control mice were inoculated *iv* with 50 – 75 µl of clinical isolates of HBV ($1x10^7$ genome equivalent copies) or control (human infectious disease free serum). HBV infected animals were treated with MGBG (50 mg/kg) at 8 weeks post infection, once a week for 5 weeks; animals were sacrifice at 16 weeks post infection to examine infection and liver disease.

[0075] Blood and tissue analysis of A2/NSG/Fas-humanized mice: Paraffin embedded fixed liver sections were stained with hematoxylin and eosin (H&E), sirius red/fast green (fibrosis), or with antibodies: anti-HBsAg (1:100; Thermo Scientific), anti-human CD68 (1:250; Dako), anti-human CD163 (1:250; Abcam), anti-human iNOS (1:250; Abcam). Immuno-reactivity was determined by incubation with DAB substrate (Pierce) or Vulcan red (Dako), and counterstained with hematoxylin (Jiang *et al., Blood 112*:2858 (2008); Zhang *et al., Blood 109*:2978 (2007)).

[0076] Human monocyte/macrophage culture: PBMC were isolated from the buffy coats of healthy HIV-1/HBV/HCV sero/qPCR negative blood donors by Ficoll-paque density gradient centrifugation. The cells were then washed, resuspended in RPMI containing pen/strep (1%), glutamine (1%), heat-inactivated FBS (10%), and seeded into tissue culture plates. Non-adherent cells, mostly T lymphocytes, were removed by gentle pipette aspiration after 1.5 h of incubation at 37°C in a humidified atmosphere containing 5% CO_2. An equal volume of fresh complete medium was

then added to each flask and attached cells at approximately 70-80% confluency were cultivated for 6 additional days at 37°C in 5% CO_2 in the presence of either rHuGM-CSF (100 ng/ml) (M1) or rHuM-CSF (100 ng/ml) (M2) for differentiation into polarized M1 or M2-monocyte derived macrophages. THP1 cells were cultured in RPMI containing pen/strep (1%), glutamine (1%), heat-inactivated FBS (10%). Polarized/differentiated primary monocyte derived macrophages and THP1 cells were washed and treated with medium containing HBV (HBV positive HepG2 cell line - HepG2.2.15 supernatant or human patient derived at MOI = 10) or Mock (HBV negative HepG2 cell line - HepG2 supernatant derived) for 6 days; cells were treated with MGBG (1 μM) or DFMO (1 mM) at the time of virus inoculation. M1 and M2 macrophage activation were examined using cytokine analysis (BD Biosciences) and qPCR (Invitrogen) following the manufacturers' recommended procedures.

[0077] Liver fibrogenesis co-culture model using human hepatic stellate (LX2) cell line: Human hepatic stellate cell line (LX2) (~10^4 cells per well – 24 well plate) was cultured for 6 days in the absence or presence of HBV and in the absence or presence of THP1 cells (~$2x10^4$ cells per well – 24 well plate) with or without polyamine synthesis inhibitor treatment; hepatic stellate cell expansion was examined using microcopy and hemocytometer.

[0078] Statistical analysis: We used unpaired two-tailed Student's t-tests or ANOVA for all comparisons. $p<0.05$ is considered significant. All data are reported as means ± standard error.

EXAMPLE 2

Polyamine inhibitor attenuates HBV–induced M2-like macrophage activation

[0079] It was previously demonstrated that HBV induces M2-like macrophage activation in human macrophages. Induction of polyamine synthesis is a major biosynthetic pathway in M2-like macrophage activation, and distinguishes the M2-like macrophage lineage from the M1 macrophage lineage which induces the production of nitrite oxide. Critical enzymes involved in M2-like macrophage polyamine synthesis include ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (SAMDC), which are pharmaceutically inhibited using D,L-alpha-difluoromethylornithine (DFMO) and methylglyoxal bis(guanylhydrazone) (MGBG), respectively. To examine the therapeutic effect of

polyamine synthesis inhibitors on HBV–induced M2-like macrophages, THP1 cells and primary M1 and M2 macrophages were incubated with HBV in the presence or absence of MGBG or DFMO (**FIGS. 1A, 1B, 1C**). It was confirmed that HBV induces M2-like macrophage activation in a human monocytic cell line (THP1) and primary macrophages as measured using gene expression and cytokine analysis respectively (**FIGS. 1A, 1B, 1C**). Importantly, both MGBG and DFMO inhibited the induction of M2-like macrophage activation in both THP1 cells and human M1 and M2 polarized macrophages as measured by gene expression and cytokine profile analysis (**FIGS. 1A, 1B, 1C**). Furthermore, MGBG enhanced M1 activation in primary polarized macrophages (**FIG. 1B**); this effect was also observed with DFMO treatment (**FIG. 1C**).

EXAMPLE 3

Polyamine synthesis inhibitors attenuate HBV-activated monocyte/macrophage induced hepatic stellate cell activation

[0080] Several studies have suggested a critical role for monocyte/macrophage/kupffer cells in the modulation of hepatic stellate cell activation. Several studies have demonstrated that M2-like macrophages promote tissue fibrosis. To examine the effect of HBV activated macrophages on hepatic stellate cell activation/expansion, LX2 cells and HepG2.2.15 cells (HBV^+ liver cell line) were co-cultured in the presence or absence of THP1 cells. Analysis of hepatic stellate cell (LX2) activation in the presence of HepG2.2.15 with or without THP1 cells showed that the presence of THP1 cells promotes hepatic stellate cell activation and expansion. Co-culturing THP1 cells with LX2 cells resulted in a dose dependent inhibition of LX2 expansion. Additionally, culturing LX2 cells in the presence or absence of HBV showed HBV does not affect hepatic stellate cell activation; however, M2 polarized primary macrophages activate LX2 cells and this effect was enhanced by HBV. As demonstrated earlier, polyamine synthesis inhibitor attenuates HBV-induced M2-like macrophage activation; thus the effect of polyamine synthesis inhibitors on hepatic stellate cell activation was examined with or without HBV inoculation and in the presence and absence of human monocytes/macrophages. Treatment of LX2 cells monoculture or THP1 co-culture with MGBG or DFMO in the presence or absence of HBV resulted in significant inhibition of hepatic stellate

cell expansion (**FIGS. 2A, 2B**). This confirmed that HBV activated THP1 cells promote LX2 cell expansion, as HBV inoculation promoted LX2 expansion in THP1 plus LX2 co-cultures compared to mock culture which exhibited reduced LX2 cell growth (**FIGS. 2A, 2B**).

EXAMPLE 4

Polyamine inhibitor attenuates HBV–induced M2-like macrophage activation and inhibit HBV persistence and associated liver diseases *in vivo*

[0081] It was previously demonstrated that chronic HBV infection induces M2-like macrophage activation in humanized mice livers, and that M2-like macrophage levels strongly correlated with liver disease. To determine the functional role of M2-like macrophage in HBV–induced immune impairment, persistent infection and liver disease *in vivo*, chronic HBV infected A2/NSG-hu HSC/Hep mice with liver disease were treated with the polyamine synthesis inhibitor MGBG at 8 weeks post inoculation for 5 weeks. To examine HBV-induced M2-like macrophage infiltration in the liver, animals were sacrificed at approximately 16 weeks post inoculation. Human macrophages of predominately M2-like lineage ($hCD68^+$ $hCD163^+$ $hiNOS^-$) were detected in the livers of vehicle treated chronic HBV infected humanized mice, but not in the livers of MGBG treated chronic HBV infected humanized mice and control animals (non-transplanted mice inoculated with HBV and mock inoculated humanized mice) (**FIG. 3**). Importantly, HBV surface antigens were detected in the livers of vehicle treated HBV infected humanized mice, but not in the livers of control animals (non-transplanted mice inoculated with HBV and mock inoculated humanized mice); a relatively low level of viral antigen was detected in the livers of MGBG treated–chronic HBV animals (**FIG. 4**). Additionally, HBV–induced chronic liver inflammation and associated liver fibrosis and damage were detected in the livers of vehicle treated chronic HBV infected humanized mice, but not in the livers of MGBG treated chronic HBV infected humanized mice and control animals (non-transplanted mice inoculated with HBV and mock inoculated humanized mice) (**FIG. 4**).

[0082] In chronically infected patients, immune and inflammatory responses against HBV are implicated as the major mediators of liver diseases (Mitchell *et al., PLoS One 6*:e27717 (2011); Kim *et al., Antivir. Ther. 16*:1169 (2011)). Additionally,

several studies have demonstrated that, despite the massive inflammatory response associated with chronic HBV infection, the Th1 immune response is impaired. Several studies have demonstrated that chronic HBV clearance and associated liver disease resolution is associated with the restoration of robust anti-viral Th1 immune response. It was recently demonstrated chronic HBV infection in the liver of A2/NSG/Fas-hu mice. Additionally, it was demonstrated chronic HBV infection in humanized mice was associated with significant human leukocyte infiltration, leading to human hepatic stellate cell activation and human liver fibrosis. Several reports have shown macrophage activation/polarization plays a critical role in modulating pathogen clearance, chronic inflammation and associated tissue fibrosis and damage; with M1 polarized macrophages promoting anti-virus Th1 immune response and pathogen clearance, while M2 polarized macrophages impair Th1 immune response and promoting tissue remodeling (Braga *et al., Mol. Med. 18*:1231 (2012); Glim *et al., Immunobiology 218*:924 (2013); Pereira *et al., Liver Int. 33*:149 (2013); Kurahara *et al., Pancreas 42*:155 (2013); Shirabe *et al., Surg. Today 42*:1 (2012)). M2 macrophages are critical innate immune cells involved in tissue remodeling/wound repair, secreting anti-inflammatory cytokines and redistributing micronutrients to sites of wound repair; however, during chronic infection, M2 macrophages promote tissue fibrosis and impair Th1 response, thus promoting pathogen persistence and associated tissue pathology (Murray *et al., Nat. Rev. Immunol. 11*:723 (2011)). It was recently demonstrated that liver inflammation and immune impairment in chronic HBV infected humanized mice livers was associated with M2-like macrophages, which also localized to fibrotic regions. Most importantly, this result was confirmed in chronic HBV and acute HBV-induced liver failure patients. Additionally, it was demonstrated that HBV promotes M2 macrophage polarization in human M1 and M2 macrophages. The present results confirmed those studies by demonstrating that HBV induces M2-like macrophage activation in the human monocytic cell line (THP1) and primary macrophages. Additionally, it was demonstrated that HBV–induced macrophage activation was associated with hepatic stellate cell activation in cell culture models.

[0083] Polyamine synthesis is a major biosynthetic pathway in M2-like macrophage activation with various enzymes including ODC and SAM playing critical roles in this pathway. It was demonstrated that administration of polyamine

synthesis inhibitors attenuates HBV–induced M2-like macrophage activation and promotes M1 macrophage activation in human macrophages. Furthermore, administration of polyamine synthesis inhibitors to chronic HBV infected humanized mice also inhibited HBV infection and associated HBV–induced chronic liver inflammation and fibrosis; this response was associated with the clearance of M2-like macrophages. These results suggest a therapeutic potential for treating HBV infection and associated liver diseases with polyamine inhibitors or other therapies targeting M2-like macrophages.

[0084] The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

That which is claimed is:

1. A method of inhibiting activation of M2-like macrophages in a subject, comprising delivering to the subject an effective amount of a polyamine synthesis inhibitor, thereby inhibiting activation of M2-like macrophages in the subject.

2. A method of decreasing the number of M2-like macrophages in a subject, comprising delivering to the subject an effective amount of a polyamine synthesis inhibitor, thereby decreasing the number of M2-like macrophages in the subject.

3. A method of inhibiting liver inflammation and/or fibrosis in a subject in need thereof, comprising inhibiting the activation of M2-like macrophages in the subject, thereby inhibiting liver inflammation and/or fibrosis in the subject.

4. A method of inhibiting liver inflammation and/or fibrosis in a subject in need thereof, comprising decreasing the number of M2-like macrophages in the subject, thereby inhibiting liver inflammation and/or fibrosis in the subject.

5. A method of treating liver disease in a subject in need thereof, comprising inhibiting the activation of M2-like macrophages in the subject, thereby treating liver disease in the subject.

6. A method of treating liver disease in a subject in need thereof, comprising decreasing the number of M2-like macrophages in the subject, thereby treating liver disease in the subject.

7. The method of any one of claims 3-6, comprising delivering to the subject a therapeutically effective amount of a polyamine synthesis inhibitor.

8. The method of any one of claims 5-7, wherein the liver disease is a chronic inflammatory liver disease.

9. The method of any one of claims 5-7, wherein the liver disease is selected from the group consisting of hepatitis virus infection, hepatocellular carcinoma, cirrhosis, fibrosis, non-alcoholic fatty liver disease, alcoholic liver disease, alcohol- or drug-induced hepatitis, organ transplant rejection, veno-occlusive disease, sinusoidal obstruction syndrome, steatohepatitis, autoimmune hepatitis, haemochromatosis, cholangiocarcinoma, metastatic cancers, Wilson's disease, Crigler-Najjar syndrome, primary sclerosing cholangitis, primary biliary cirrhosis, Budd-Chiari syndrome, protoporphyria, Gilbert's syndrome, rotor syndrome, glycogen storage disease type 2, hemangioma, hyperbilirubinemia, biliary atresia, Byler disease, Dubin-Johnson syndrome, alpha-1 antitrypsin deficiency, Caroli disease, Alagille syndrome, progressive familial intrahepatic cholestasis, and any combination thereof.

10. The method of any one of claims 1-9, wherein the polyamine synthesis inhibitor is an inhibitor of an enzyme in the polyamine synthesis pathway.

11. The method of claim 10, wherein the inhibitor is α-difluoromethylornithine, α-methylornithine, monofluoromethyldehydroornithine methylester, α-difluoromethylarginine, methylglyoxal bis(guanylhydrazone), (N^1,N^{12}-bis-(ethyl)-spermine, 1,19-bis-(ethylamino)-5,10,15, triazononadecane, or sardomozide.

12. The method of any one of claims 1-11, further comprising delivering to the subject a liver disease therapeutic agent.

13. The method of any one of claims 1-12, wherein the number of M2-like macrophages in the subject is decreased by at least about 50%.

14. The method of any one of claims 1-13, wherein the number of M2-like macrophages in the subject is decreased by at least about 80%.

15. The method of any one of claims 1-14, wherein the subject is a human.

16. The method of any one of claims 1-14, wherein the subject is an animal model of liver disease.

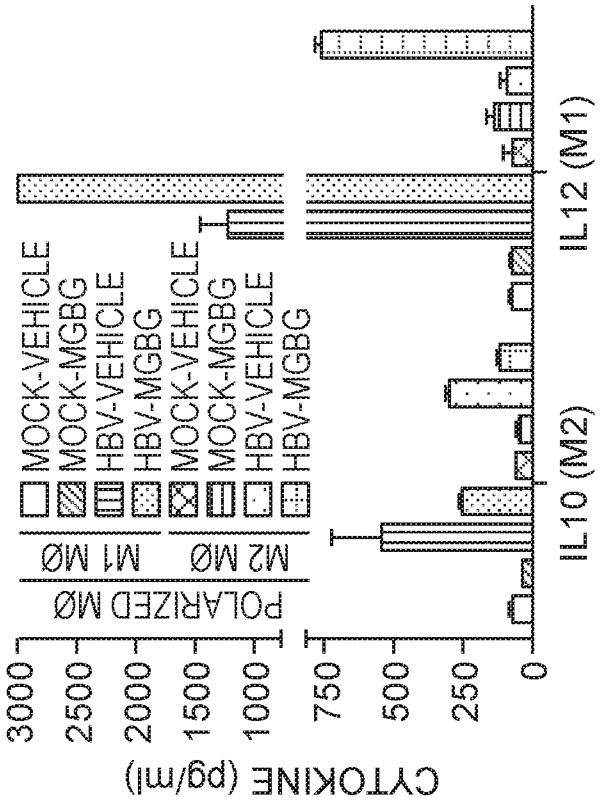

FIG. 1A

FIG. 1B

FIG. 1C

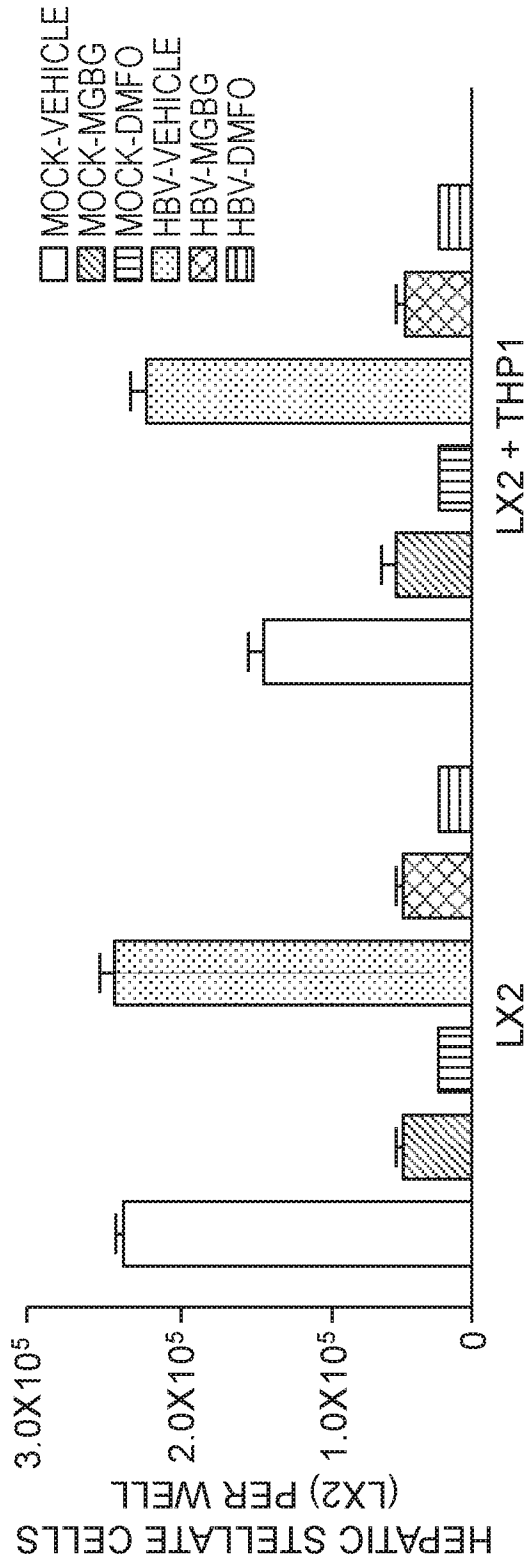
MOCK-VEHICLE
MOCK-MGBG
MOCK-DMFO
HBV-VEHICLE
HBV-MGBG
HBV-DMFO
HEPATIC STELLATE CELLS (LX2) PER WELL
3.0X10^5
2.0X10^5
1.0X10^5
0
LX2
LX2 + THP1

FIG. 2A

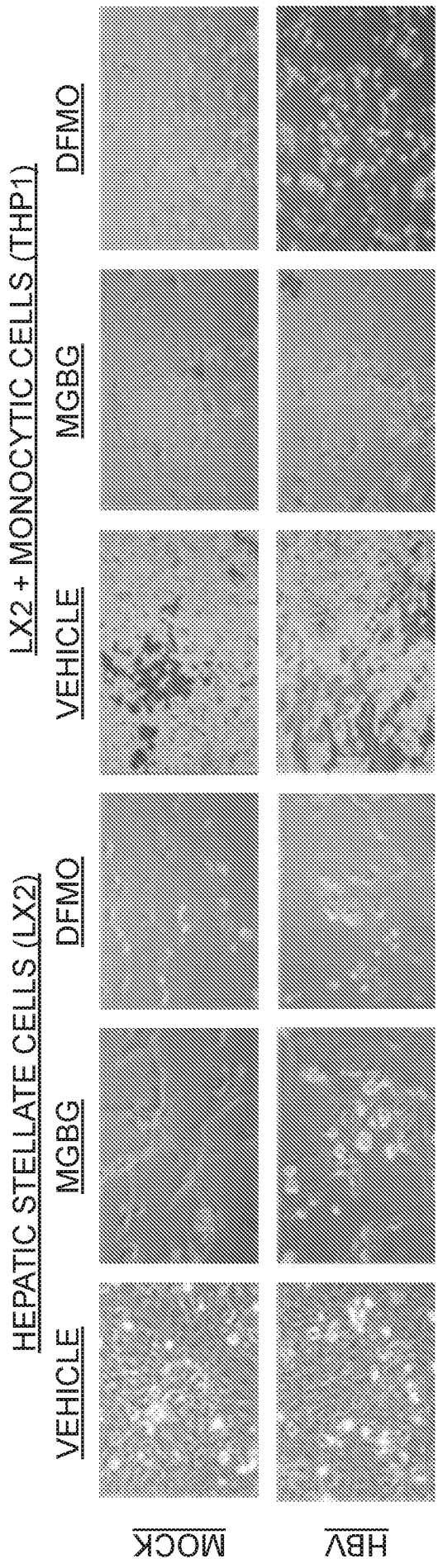
HEPATIC STELLATE CELLS (LX2)
LX2 + MONOCYTIC CELLS (THP1)
VEHICLE
MGBG
DFMO
VEHICLE
MGBG
DFMO
MOCK
HBV

FIG. 2B

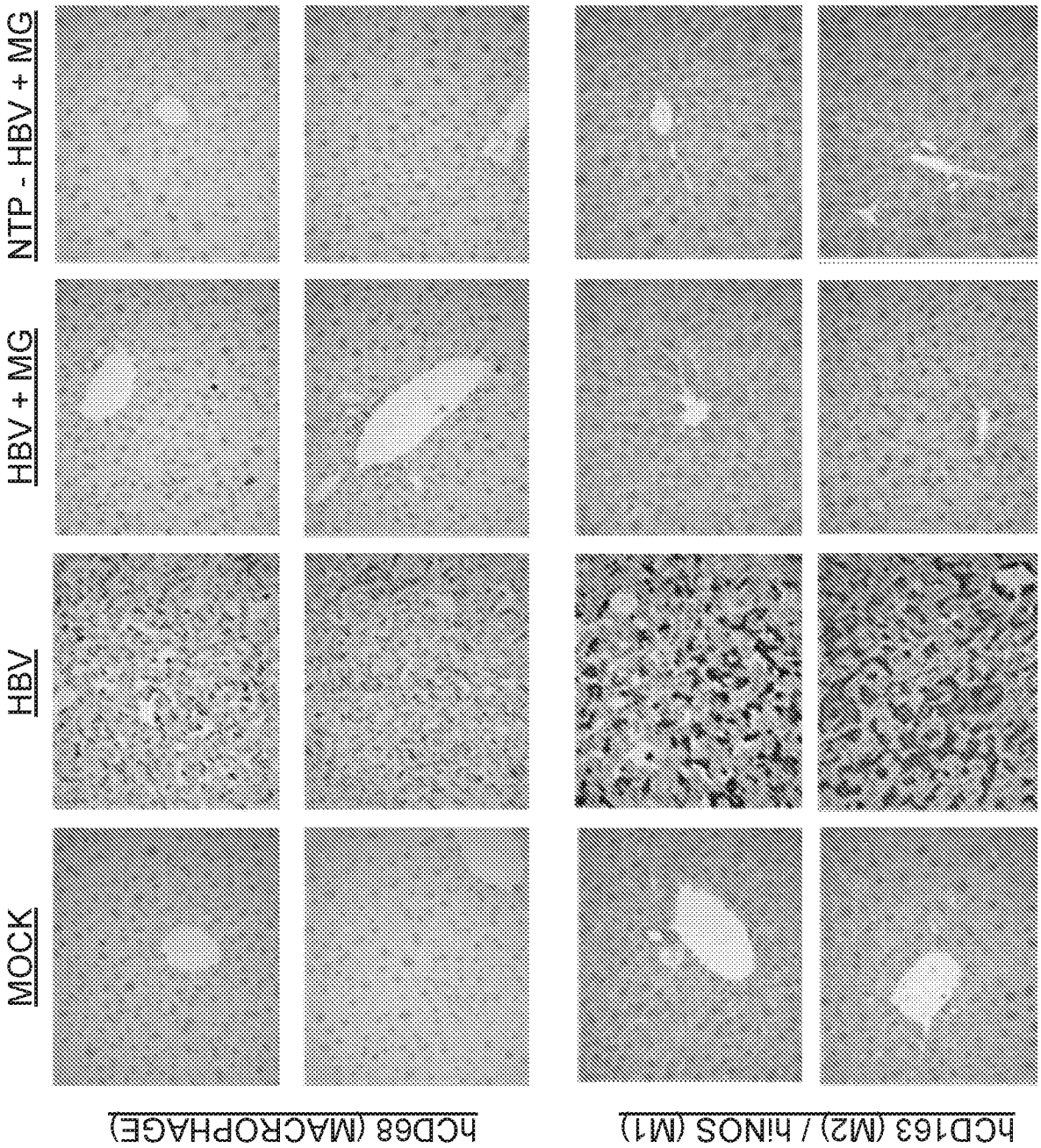
MOCK
HBV
HBV + MG
NTP - HBV + MG
hCD68 (MACROPHAGE)
hCD163 (M2) / hiNOS (M1)

FIG. 3

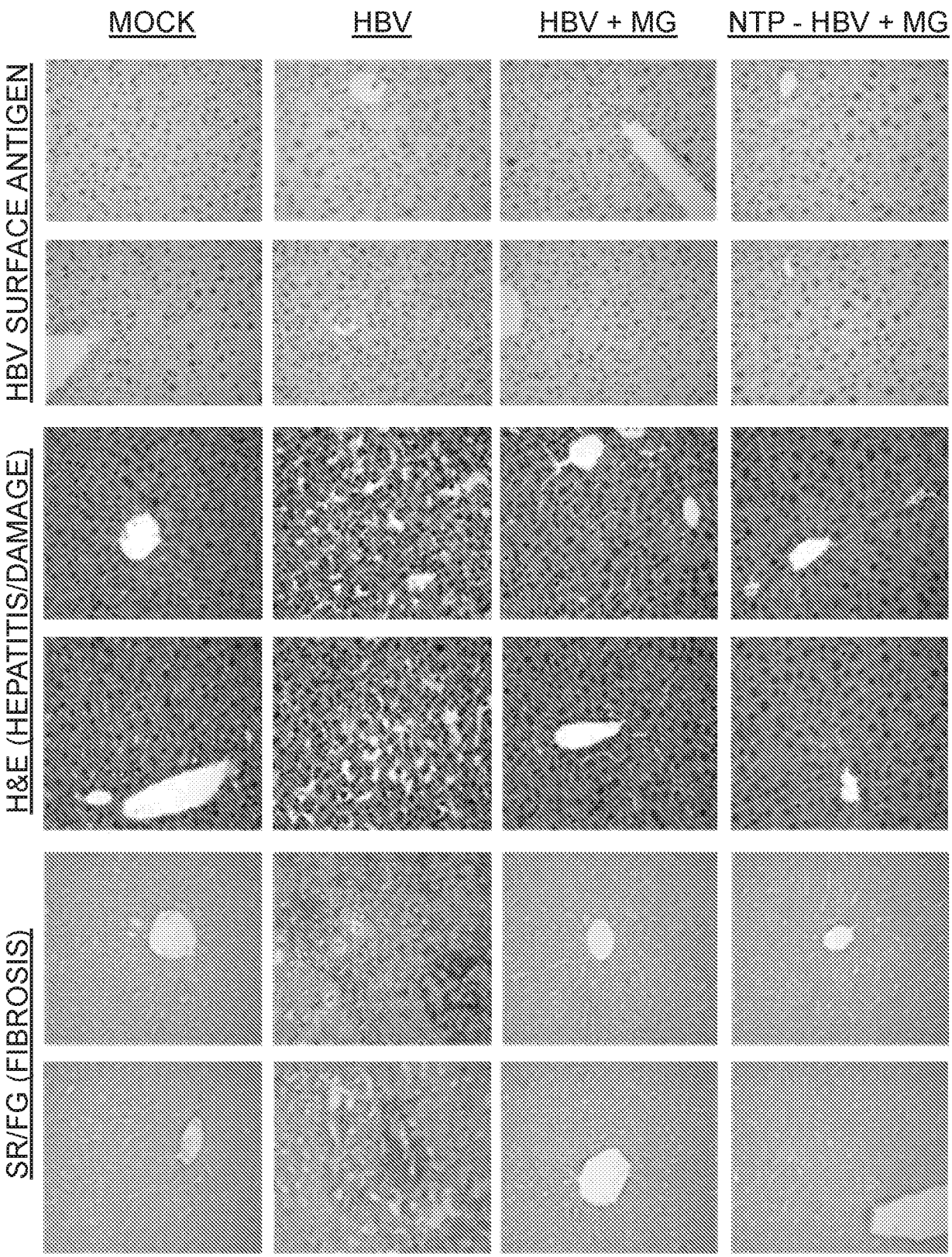
MOCK
HBV
HBV + MG
NTP - HBV + MG
HBV SURFACE ANTIGEN
H&E (HEPATITIS/DAMAGE)
SR/FG (FIBROSIS)

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2016/031984

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/197 *(2006.01)*
A61P 1/16 *(2006.01)*

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)

A61K 31/197, 31/195, 31/19, 31/185, 31/00, A61P 1/16, 1/00

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

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

EAPATIS, RUPAT, Patentscope, DWPI, NCBI (PubMed), SpringerLink

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	BILITY Moses T. et al. Hepatitis B Virus Infection and Immunopathogenesis in a Humanized Mouse Model: Induction of Human-Specific Liver Fibrosis and M2-Like Macrophages. PLOS Pathogens, 2014, Vol. 10, Issue 3, e. 1004032	3-6 1-2, 7
Y	CASERO Robert A. et al. Polyamine catabolism and disease. Biochem J., 2009, 421(3):pp. 323-338	1-2, 7
A	ZIRVI K.A. et al. Alpha-Difluoromethylornithine inhibits liver metastasis produced by intrasplenic injection of human tumor cells into nude mice. Clin Exp Metastasis, 1989, 7(6):pp. 591-598 (abstract) [online] [retrieved on 08.08.2016] Retrieved from PubMed, PMID:2505959	1-7

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"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 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
29 July 2016 (29.07.2016)	29 September 2016 (29.09.2016)
Name and mailing address of the ISA/RU: Federal Institute of Industrial Property, Berezhkovskaya nab., 30-1, Moscow, G-59, GSP-3, Russia, 125993 Facsimile No: (8-495) 531-63-18, (8-499) 243-33-37	Authorized officer D. Rubaylo Telephone No. (495)531-64-81

Form PCT/ISA/210 (second sheet) (January 2015)