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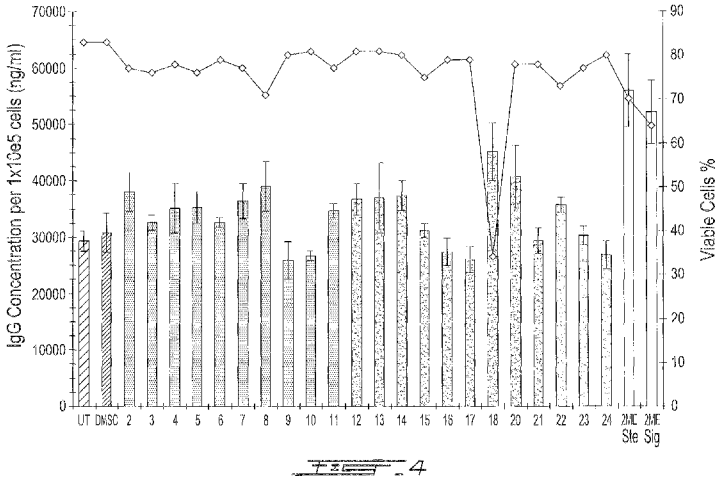

Fig. 4

(57) Abstract: The present application relates to the use of a steroidal compound for the conversion of B lymphocytes into plasma cells and/or for the production of *in vitro* polyclonal antibody compositions. The present application also relates to the use of the polyclonal antibody compositions described herein for the treatment of immune deficiencies and/or other immune conditions.

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USE OF STEROIDAL COMPOUNDS FOR THE CONVERSION OF NORMAL B LYMPHOCYTES INTO IMMUNOGLOBULIN-SECRETING PLASMA CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. provisional patent application 61/222,985 filed on July 3, 2010 and incorporated herewith in its entirety.

BACKGROUND

[0002] Intravenous Igs (IVIg) are prepared from pooled plasmas of healthy donors and are composed of 95-98% IgG. IVIg are used to passively protect immunodeficient individuals against infectious diseases. These IgG preparations include a large repertoire of human IgGs reacting against self- and non-self-antigens. IVIg are also widely used for the treatment of autoimmune and inflammatory diseases, including neurological disorders. Autoimmune and inflammatory diseases are characterized by an uncontrolled activity of the immune system, and treatment with IVIg has been shown to restore a normal immune equilibrium in many clinical situations. An increasing number of studies are suggesting new indications for IVIg, resulting in a continuous increase in their utilization, and raising the possibility of product shortages with concomitant increased costs because of the higher demand.

[0003] IgGs are secreted by plasma cells, which are generated following antigenic activation of B lymphocytes in the presence of auxiliary cells. Naïve and memory B lymphocytes are both CD19+/CD138- and can be distinguished mainly by the presence of CD27 specifically on memory B cells, whereas plasma cells can be distinguished from B lymphocytes by the presence of CD138. Both naïve and memory B lymphocytes can be activated following antigenic binding of their specific surface Igs, also referred to as B-cell antigen (Ag) receptors (BCR). Ag-activated naïve and memory B lymphocytes, however, require supplemental interactions with auxiliary cells and soluble factors to survive and proliferate. Ag-activated naïve B lymphocytes can

differentiate into short-lived plasma cells or migrate to germinal centers in lymphoid secondary organs where affinity maturation of their BCR will allow the emergence of memory cells carrying antigenic receptors with increased affinity. Following a second antigenic challenge, memory B lymphocytes will differentiate into plasma cells and secrete antibodies of higher affinity. Notably, these IgG+ memory B lymphocytes that have the potential to become IgG-secreting plasma cells represent less than 15% of all B cells found in human peripheral blood.

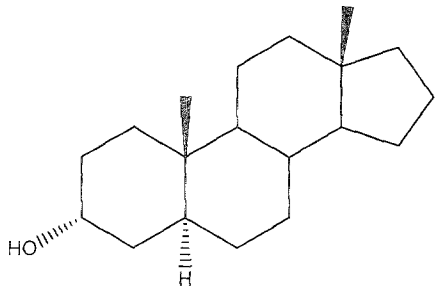
[0004] A very significant advance in the *in vitro* culture of human B lymphocytes was the finding that the CD40 receptor expressed on B lymphocytes was providing a crucial stimulatory signal after activation by gp39, a T-cell ligand that has since been renamed CD154. This interaction is central to the T-cell-dependent proliferation of antigen-activated B lymphocytes but also to the generation of memory B lymphocytes. From this discovery, Banchereau et al. developed a culture system allowing long-term proliferation of B lymphocytes. A similar CD40 system based on the utilization of a membranous form of CD154 to stimulate the proliferation and differentiation of naïve and memory B lymphocytes is also being used. It has also been shown that the CD40–CD154 interaction behaves like a rheostat, directing B lymphocytes toward differentiation or proliferation. For instance, a low-intensity CD154 signal may induce differentiation towards memory B cells, whereas a stronger-intensity signal induces preferential proliferation of naïve B cells. Despite the deeper knowledge of B-cell biology, the *in vitro* conversion of naïve and memory B lymphocytes into IgG-secreting plasma cells remains a major challenge.

[0005] It would be highly desirable to be provided with a method of increasing the conversion of B cells into plasma cells. This conversion could be useful for the *in vitro* production of antibodies from normal B lymphocytes.

BRIEF SUMMARY

[0006] An aim of the present invention is to provide a method for increasing the conversion of B lymphocytes (such as memory B cells) into Ig-secreting plasma cells and/or generating polyclonal antibodies *in vitro*.

[0007] In a first aspect, the present application provides a method of inducing the conversion of a normal B lymphocyte into a plasma cell. Broadly, the method comprising exposing the normal B lymphocyte to a steroidal compound, thereby favoring the conversion of the normal B lymphocyte into a plasma cell. In an embodiment, the steroidal compound has the formula of any one of compounds 2, 4, 5, 8, 9, 10, 11, 12, 13, 14, 18, 19, 21 and 23 as set forth in Table 4. In another embodiment, the steroidal compound has the formula:



[0008] In still a further embodiment, wherein the exposure occurs *in vitro*. In yet another embodiment, the steroidal compound is added to a culture medium for the culture of the normal B lymphocyte. In still another embodiment, the method further comprises exposing an initial population of B lymphocytes comprising the normal B lymphocyte to the steroidal compound to provide a cultured population of B lymphocytes. In yet another embodiment, the initial population of B lymphocytes comprises CD19+ cells, and in a further embodiment, at a concentration higher than about 90% or 95%. In still a further embodiment, the initial population of B lymphocytes comprises CD27+ cells. In another embodiment, the B lymphocytes of the initial population are normal B lymphocytes. In an embodiment, the cultured population of B lymphocytes comprises CD138+ cells, and in a further embodiment, . the percentage of CD138+ cells in the cultured population of B lymphocytes is at least about 10.0%, 15.0%, 20.0%, 25.0%, 30.0%, 35.0% or 40.0%. In another embodiment, the method comprises isolating CD138+ cells from the cultured population of B lymphocytes. In still another embodiment, the method comprises culturing the isolated CD138+ cells.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] Fig. 1. Overall Experimental Strategy for Normal Human B-Lymphocyte Culture, Treatment, and Sample Collection. B lymphocytes harvested from a healthy individual were cultured according to the method described in Example I. Untreated B lymphocytes were maintained in culture for up to 15 days. On days 5, 7, 10, and 12, aliquots of this culture were collected and treated for three days with either DMSO or a steroidal compound. At the end of treatment, cultures were harvested and assessed for proliferation, viability, and percent CD138 expression, and compared to the untreated culture sampled on the same days.

[0010] Fig. 2. Polyclonality of Secreted IgG. Cell supernatants used for the ELISA results presented in Example II were subjected to isoelectrofocusing, as described in Example I. Supernatants from untreated B lymphocytes (UT), B lymphocytes treated with 0.75 µM 2ME (2ME) and CD138+ cells purified from 2ME-treated B lymphocytes (CD138) were analyzed. An IEF marker (Marker) and a control, purified, in-house human monoclonal antibody (CT) were run in parallel.

[0011] Fig. 3. IgG Transcript Subclass Analysis by RT-PCR. Total RNA from untreated B lymphocytes, 2ME-treated B lymphocytes and CD138+ cells purified from 2ME-treated B lymphocytes was isolated and subjected to RT-PCR using primers specific for either IgG1, IgG2, IgG3 or IgG4. RT-PCR products were resolved on a 1% agarose gel stained with GelRed 30™. Lanes correspond to untreated B lymphocytes (1), 2ME-treated B lymphocytes (2), CD138+ cells purified from 2ME-treated B lymphocytes (3), negative control without RNA (4) and molecular weight marker (5).

[0012] Fig. 4. B-Lymphocyte Viability and Ig Secretion in Response to Various Steroids. B lymphocytes cultured for five days were either maintained untreated for an additional three days or treated for three days with 0.75 µM of the indicated steroid. Viability was assessed by counting viable cells with a haemacytometer after Trypan blue dye staining. Secreted IgG concentration was determined by ELISA in cell supernatants. Results are expressed in ng

IgG/ml/10^5 cells, and represent means +/- standard deviations of triplicate treatments.

[0013] Fig. 5 Polyclonality of Secreted IgG. Cell supernatants used for the ELISA results presented in Example II were subjected to isoelectrofocusing, as described in Example I. Supernatants from untreated B lymphocytes (ut), B lymphocytes treated with 0.25 µM 5α-Androstan-3α-ol and CD138+ cells purified from 5α-Androstan-3α-ol -treated B lymphocytes (CD138) were analyzed. A control corresponding to a monoclonal human antibody (ct), and an IEF marker were run in parallel.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0014] In accordance with the present invention, there is provided the use of steroidal compounds to favor the conversion of normal B cells into plasma cells and/or increase the *in vitro* production of a polyclonal antibody composition. The polyclonal antibody composition produced herein can be used in the treatment of various immune conditions where IVIg are currently being administered.

[0015] 2-Methoxyestradiol (2ME), an end-metabolite of 17β-estradiol of very low affinity for the estrogen receptor, has been reported as a promising anti-cancer drug. In particular, 2ME has been advocated as a possible treatment option for multiple myeloma, a neoplastic disorder of terminally differentiated CD138+ plasma cells. Some studies have shown that 2ME induces apoptosis and also suppresses proliferation of multiple myeloma cells. Other anticancer mechanisms have been suggested, including inhibition of tubulin polymerization and of superoxide dismutase activity. Moreover, 2ME has also been described as an inhibitor of angiogenesis and suppressor of tumor growth. However, the effects of 2ME on normal human peripheral blood cells, if they exist, have not yet been reported. As shown herein, steroidal compounds, such as 2ME, are capable of increasing the conversion of B cells into plasma cells.

[0016] The exposure of the steroidal compound favors the conversion of a portion of the original B cell population (mostly being CD138- B lymphocytes) into plasma cells. As such, there is provided herein a method of increasing the

conversion of normal B lymphocytes into plasma cells. In an embodiment, the percentage of plasma cells in the population of cells exposed to the steroidal compound is at least 10.0%, at least 15.0%, at least 20.0% or at least 25.0%.

[0017] Optionally, the method can also comprises isolating and/or culturing the CD138+ cells produced by the method. The culture of the isolated CD138+ cells can be used, for example, for the production of a polyclonal antibody composition *in vitro*.

[0018] The methods described herein are applied to B lymphocytes. As used herein, the term "B lymphocyte" or "B cell" refers to lymphocytes that play a role in the humoral immune response and is a component of the adaptive immune system. In this application, the expressions "B cell", "B-cell" and "B lymphocyte" refer to the same cell. B cells can be isolated from a primary explant containing B cells or from an extract. B cells are usually isolated from the spleen, tonsils, bone marrow or peripheral blood of mammals. In an embodiment, the subject from which the B cell is isolated has been previously put in contact with a specific antigen or epitope and has produced B-cell clones specific to that antigen or epitope. The present method also contemplates the use of B cells directly isolated from a subject (e.g. such as "raw" extracts) or cultures of B cells derived from such isolates.

[0019] B cells can be obtained from various sources, for example, human or other primate, rodent (including rat or mouse), horse, cow, dog, cat, pig, goat, sheep, llama, camel, dromedary, or rabbit. Alternately, B cells can be obtained from an avian source, such as a chicken, turkey, duck or goose. B cells could also be derived from a reptile such as a snake, crocodile or turtle, or a fish such as a carp or a shark. In an embodiment, B cells are initially exposed to an antigen or pathogen of interest prior to or subsequent to the method presented herein.

[0020] The B lymphocyte population used herein in the methods described herein is considered to be "normal" because their cell cycle is adequately regulated. The term "normal" is used to contrast with the term "malignant" or

"cancerous" B cells. The term "normal" refers, for example, to B cells derived or isolated from a subject who is not experiencing cancer, such as leukemia, lymphoma or myeloma.

[0021] The B cells can be optionally purified prior to or subsequent to the methods described herein. In some instances, it may be advantageous to select CD19+ cells from a B cell extract or culture prior to submitted them to the methods described herewith. Alternatively or optionally, it may be advantageous to select CD138+ cells from the B cell population obtained after exposure to the steroidal compound.

[0022] The methods provided herewith can also be used on a "population of B lymphocytes". This term refers to a population of B cells that contains more than one B cell clone and/or a polyclonal B cell preparation. As it is currently known in the art, B cells exist as clones, e.g., each B cell is derived from a unique precursor, and thus, the antibodies that their differentiated progenies produce can recognize and/or bind a unique epitope. The method presented herein contemplates the use of B cells that can be derived from various sources.

[0023] Immature or naïve B cells are produced in the bone marrow of most mammals. After reaching the IgM+ immature stage in the bone marrow, these immature B cells migrate to the lymphoid organs, where they are referred to as transitional B cells, some of which subsequently differentiating into mature B lymphocytes. B-cell development occurs through several stages, each stage characterized by a change in the genome content at the antibody loci. Immature or naïve B cells are known to express the CD19 marker and usually fail to express the CD27 or the CD138 marker.

[0024] Each B cell has a unique receptor protein (referred to as the B-cell receptor (BCR)) on its surface that is able to bind to a unique antigen. The BCR is a membrane-bound immunoglobulin, and it is this molecule that allows to distinguish B cells from other types of lymphocytes, as well as playing a central role in B-cell activation *in vivo*. Once a B cell encounters its cognate antigen and

receives an additional signal from a T helper cell, it can further differentiate into either one of two types of B cells (plasma B cells and memory B cells). The B cell may either become one of these cell types directly or it may undergo an intermediate differentiation step, the germinal center reaction, during which the B cell hypermutates the variable region of its immunoglobulin gene ("somatic hypermutation") and possibly undergoes class switching.

[0025] Plasma cells (also known as plasma B cells or plasmocytes) are large B cells that have been exposed to an antigen and are producing and secreting large quantities of antibodies. These are short-lived cells and usually undergo apoptosis when the agent that induced the immune response (e.g. antibody production) is eliminated. Plasma cells can be recognized by their ability to produce antibodies. In addition to the expression of CD19+, plasma cells are recognized by their expression of the CD138 marker.

[0026] In contrast to plasma cells, memory B cells are formed from activated B cells that are specific to an antigen encountered during a primary immune response. These cells are able to live for a long time, and can respond quickly following a second exposure to the same antigen. Memory B cells do express the CD19 marker as well as the CD27 marker but fail to express the CD138 marker. The exposure of memory B cells to the steroidal compound as shown herewith is particularly useful for the conversion of those cells into plasma cells.

[0027] A method of increasing the production of a polyclonal antibody composition of a culture of a population of normal polyclonal B lymphocytes is also provided herewith. The method comprises exposing a population of normal B lymphocytes with the steroidal compound. In the presence of the steroidal compound, the population produces higher titers of antibodies and consequently an increased amount of a polyclonal antibody composition. In this method, an increase in CD138+ cells (e.g. plasma cells) with respect to the original (untreated) population can also be observed.

[0028] As used herein, the term “polyclonal antibody composition” refers to a composition comprising at least two antibodies either having different affinity for

a particular epitope, having affinity for different epitopes and/or being from different types or subtypes of antibodies. A polyclonal antibody composition is derived from at least two distinct B cell clones. A polyclonal antibody composition differs from a monoclonal antibody composition because of the presence of variations in physico-chemical properties (e.g. affinity and/or specificity) between the antibodies present in the composition.

[0029] The polyclonal antibody composition produced can comprise antibodies from any type and every subtypes. In an embodiment, the polyclonal antibody composition comprises IgG antibodies, such as IgG1, IgG2, IgG3 and/or IgG4. In an embodiment, the rate of production of the polyclonal antibody composition is at least 20 μg IgG / 10^6 B lymphocytes / 24h. In another embodiment, the polyclonal antibody composition comprises IgM antibodies. In yet another embodiment, the rate of production of the polyclonal antibody composition is at least 1 μg IgM / 10^6 B lymphocytes / 24h.

[0030] As used herein, the term "antigen" refers to an agent to which an antibody can bind to *via* its antigen-binding sites. Antigens come in various forms and include, but are not limited to, proteins, peptides, carbohydrates, lipids, synthetic compounds and combinations thereof. An antigen comprises one or more than one epitope. An epitope, also known as an antigenic determinant, is the part of an antigen that is recognized by the immune system.

[0031] As used herein, the term "culturing" or "cultured" refers to the steps used *in vitro* to incubate a population of cells (such as a population of B lymphocytes) under conditions that support the growth, viability and/or differentiation of the cells. In the art, it is widely recognized that a number of formats, medias, temperature range, gas concentrations, culture additives, culture support etc., will support the growth, viability and/or differentiation of the cells, and that specific parameters need to be defined in the culture system of interest. The parameters of the culture will vary depending on the format selected and the specific goals of the culture. It is recognized that the determination of adequate culture parameters is routine in the art. In the

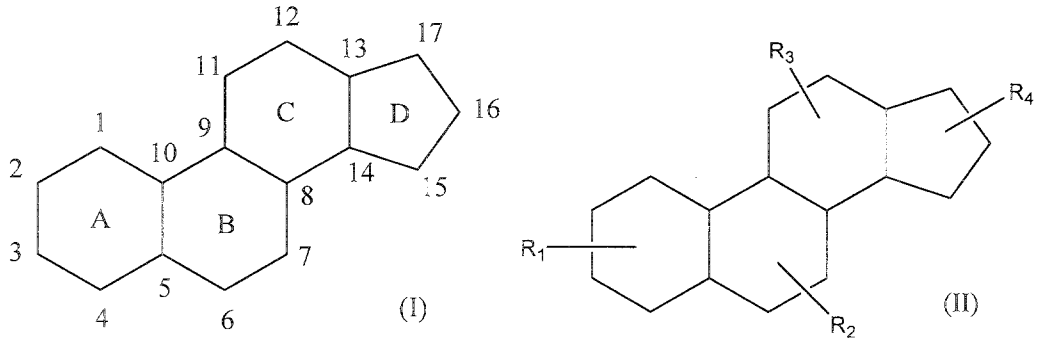
methods described herein, the parameters must be optimized for the production of a large quantity of polyclonal antibody compositions.

[0032] Such culture conditions can include, for example, adding CD154 in the culture medium to favor antibody production from the population of normal B cells. As indicated above, such an addition has been described in the art as facilitating the production of antibodies *in vitro* by a B cell population.

[0033] To determine if the culture conditions are adequate, one can monitor various parameters of the cultured B cells. In order to determine the proliferation of B cells during culture, a variety of procedures are currently known and used in the art. These method include, but are not limited to, the measure of incorporation of a labeled compound (such as tritiated thymidine, bromodeoxy uridine), direct cell counts, fluorometry-derived counts, PCR, etc. In order to determine the differentiation of B cells and their ability to produce immunoglobulins, procedures currently known and used in the art exists. These procedures include, but are not limited to, flow cytometry, ELISA assays and PCR.

[0034] An effective amount of the steroidal compound should be added to the culture medium in order to increase the antibody production/favor the conversion into plasma cells. An amount is considered "effective" if it favors the conversion into plasma cells and/or increase the production of antibodies from the cultured cells. In an embodiment, the viability of the cells submitted to the method is at least 60%, 65%, 70% or 75%. The effective amount of the steroidal compound can vary depending on the culture conditions. In an embodiment, the concentration of the steroidal compound is between about 100 pM and 1.0 μM, between about 1.0 nM and 1.0 μM, between about 10 nM and 1.0 μM, between about 100 nM and 1.0 μM, between about 0.5 μM and 1.0 μM and, in another embodiment, the concentration of the steroidal compound is about 0.75 μM.

[0035] "Steroidal compound(s)" for use in embodiments of this disclosure include compounds having the polycyclic fused A-B-C-D ring system as defined herein and represented by formula (I) and (II):



Formula (I) is illustrating the ring positions of the basic steroidal skeleton and formula (II) the substituents of rings A, B, C or D wherein each of R1 to R4 are one or more optional substituent(s). For greater certainty, R1 can be more than one substituent of the A ring, R2 can be more than one substituent of the B ring, R3 can be more than one substituent of the C ring, R4 can be more than one substituent of the D ring. Additionally, A, B, C and/or D rings optionally contains one or more unsaturation(s).

[0036] The term "steroidal compound(s)" comprises all natural, semisynthetic and synthetic steroidal compound(s), their analogs and derivatives thereof, including metabolites thereof. Those include animal steroids (which themselves includes insect and vertebrates), plant and fungus steroids. Examples of families of steroidal compound(s) comprise : androstanes such as 5-alpha-androstan-3-alpha,17-alpha-diol, 5-alpha-androstan-3-beta,17-alpha-diol, 5-alpha-androstan-3-alpha-ol, 5-alpha-androstan-3-beta-ol, 5-alpha-androstan-17-beta-ol, 5-beta-androstan-3-alpha,17-alpha-diol, 5-beta-androstan-3-beta,17-alpha-diol, 5-beta-androstan-3-beta,17-beta -diol, 5-beta-androstan-3-alpha-ol, 5-beta-androstan-3-beta-ol; bile acids such as cholic acid, deoxycholic acid, and chenodeoxycholic acid; cardanolides; cholestanes such as cholesterol, fusidic acid, lanosterol, and stigmasterol; estranes such as 17-beta-estradiol (E2), 17-beta-estradiol 3-methyl ether, 2-methoxyestrone, 1-methylestradiol, estrone (E1), 2-methoxyestradiol 3-methyl ether, 2-ethoxyestradiol, 4-hydroxyestradiol, 4-methoxyestradiol; pregnanes such as aldosterone, progesterone and cortisol.

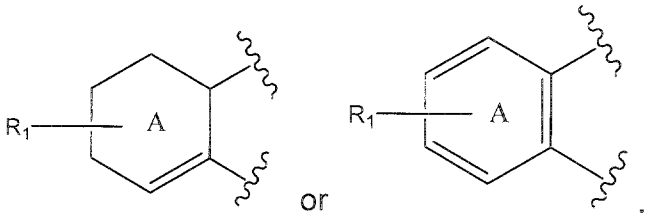
[0037] Analogs include homosteroids, in particular homosteroids of the androstane and estrane families, such as scalaradial (12-acetyloxy-4,4,8,19-tetramethyl-D-homoandrost-16-ene-17,17-adicarboxaldehyde), 16-acetoxy-17-

acetoxymethyl-11,17-dihydroxy-D-homoandrosta-1,4-diene-3,17-dione, 17-hydroxy-7-methyl-D-homoestra-4,16-dien-3-one, 6-oxabenz(3,4)-D-homoestra-1,3,5(10),8,14-pentaen-17-one, 6-hydroxy-D-homo-8-isoestrone methyl ether, 7-alpha,17-alpha-diaza-7,17-dioxo-B,D-dihomo-5-androsten-3-yl 4-N,N-bis(2-chloroethyl)aminophenylacetate; norsteroids such as desogestrel, ethinyl estradiol, ethylestrenol, ethynodiol diacetate, gestrinone, levonorgestrel, norethindrone, norgestrel and quinestrol; hydroxysteroids such as 17-hydroxypregnenolone ; steroidal sapogenins and aza-steroids.

[0038] The "steroidal compound(s)" for use in embodiments of this description can be obtained from commercial sources (such as Steraloids Inc., Newport, RI, USA) or prepared by synthesis or semi-synthesis in accordance with the chemical synthetic procedures described in the art.

[0039] It is understood by the skilled practitioner that some of the specific steroids mentioned above may be included in more than one family depending on the classification.

[0040] In one embodiment, the A ring is



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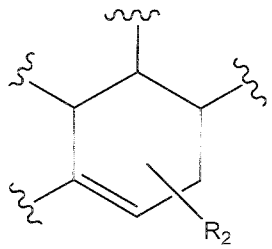
[0041] In one embodiment, the B ring is



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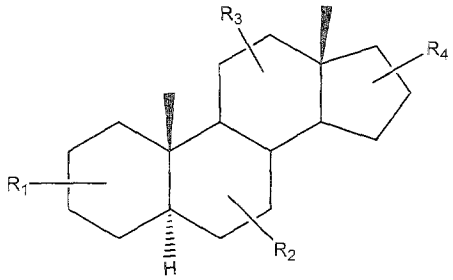
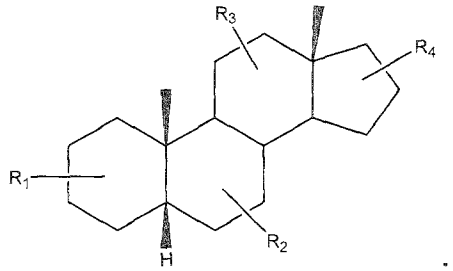
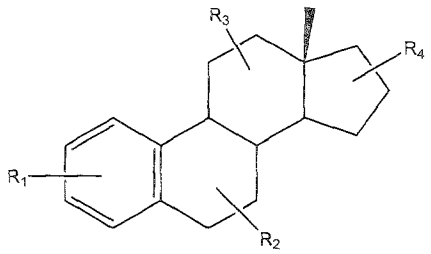
[0042] In one embodiment, the steroidal compound has the formula



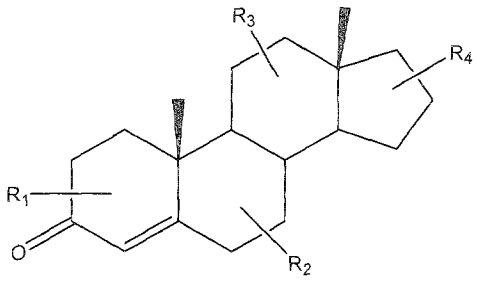
or



[0043] In one embodiment, the steroidal compound has the formula:



[0044] In one embodiment, the steroidal compound has the formula:

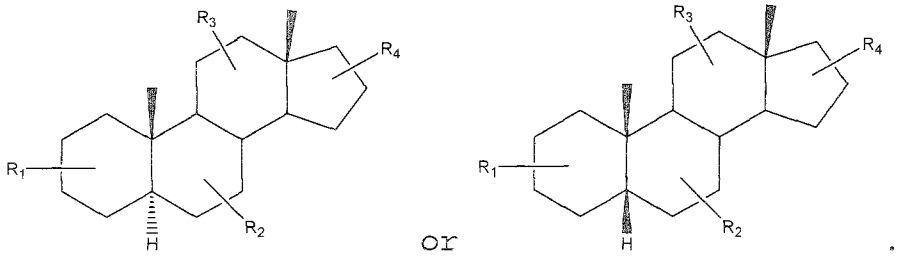


[0045] The substituents R1 to R4 are the substituents that are found in natural, semi-synthetic and synthetic steroidal compound(s), their analogs and derivatives thereof, including metabolites thereof.

[0046] In one embodiment, each of R1 to R4 is independently hydroxyl, oxo (=O), C(O)Ru (wherein Ru is selected from H, C1-6 alkyl), C1-6alkoxy or C1-6alkyl.

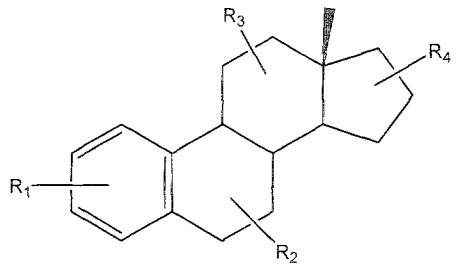
[0047] In one embodiment, each of R1 to R4 is independently hydroxyl, oxo (=O), $C(O)CH_2H$, methoxy or methyl.

[0048] In one embodiment, the steroidal compound has the formula



wherein R1 is one hydroxyl substituent, R4 is hydrogen or one hydroxyl substituent; and R2 and R3 are hydrogen. In one embodiment, R1 is C3-hydroxyl, R4 is hydrogen or C17-hydroxyl; and R2 and R3 are hydrogen.

[0049] In one embodiment, the steroidal compound has the formula:



wherein R1 is one or two substituent of position C2, C3 and C4 selected from hydroxyl and C1-6alkoxy, R4 is one substituent of position C17 selected from hydroxyl and oxo; and R2 and R3 are hydrogen.

In one embodiment, the steroidal compound has the formula of table 4 as follows: Compounds 2, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 21 and 23.

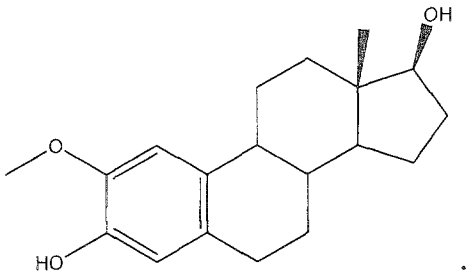
In one embodiment, the steroidal compound has the formula of table 4 as follows: Compounds 2, 4, 5 and 6.

In one embodiment, the steroidal compound has the formula of table 4 as follows: Compounds 8, 9, 10 and11.

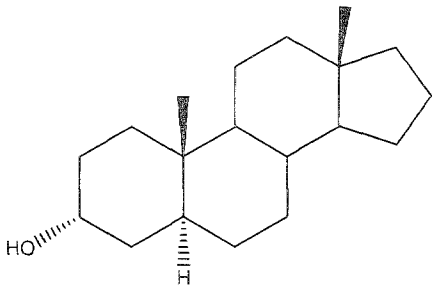
In one embodiment, the steroidal compound has the formula of table 4 as follows: Compounds 12, 13, 14, 16, 17, 18, 19 and 21.

In one embodiment, the steroidal compound has the formula of compound 23.

In one embodiment, the steroidal compound has the formula:



In one embodiment, the steroidal compound has the formula:



[0050] The term "alkyl" represents a linear or branched hydrocarbon moiety having 1 to 6 carbon atoms, which is optionally substituted. In one embodiment, the alkyl substituent is hydroxyl The term alkyl is also meant to include alkyls in which one or more hydrogen atom is replaced by a halogen.

[0051] The term "alkoxy" represents an alkyl which is covalently bonded to the adjacent atom through an oxygen atom.

[0052] Since the polyclonal antibodies composition produced by the methods described herein are similar to IVIg, they can be successfully used in the treatment of various conditions associated with immune deficiency, autoimmunity, inflammation, or neurodegenerative disorders. As such, the present application provides a method of treating an immune condition in an individual in need thereof, said method comprising administering the polyclonal antibody composition produced by the method described herein to the individual, thereby treating the immune condition. In an embodiment, the immune condition is an immune deficiency, such as a primary immunodeficiency (congenital agammaglobulinaemia, congenital

hypogammaglobulinaemia, common variable immunodeficiency, X-linked immunodeficiency with hyper IgM, severed combined immunodeficiency and/or Wiskott-Aldrich syndrome), an HIV infection, a bone marrow transplantation, a B-cell lymphocytic leukemia and a multiple myeloma. In another embodiment, the immune condition is associated with autoimmunity and/or inflammation (idiopathic thrombocytopenic purpura, Guillain-Barré syndrome, Kawasaki disease, chronic inflammatory demyelinating polyneuropathy, autoimmune neutropenia, autoimmune hemolytic anemia, anti-factor VIII autoimmune disease, multiple sclerosis, myasthenia gravis, stiff person syndrome, mutlifocal neuropathy, systemic vasculitis, polymyositis, dermatomyositis, rheumatoid arthritis, systemic lupus erythematosus, antiphospholipid syndrome, toxic epidermal necrolysis, autoimmune skin blistering disease, steroid-dependent atopic dermatitis, graft vs. host disease and/or sepsis syndrome). In another embodiment, neurodegenerative disorders are conditions associated with progressive neurodegenerescence of the central nervous system, such as Alzheimer's disease.

[0053] Prior to its administration to an individual in need thereof, the polyclonal antibody composition can be further isolated or purified. When providing an individual with the polyclonal antibody composition, the dosage of the administered polyclonal antibody composition will vary depending upon such factors as the individual's age, weight, height, sex, general medical condition, previous medical history, etc. In general, it is desirable to provide the recipient with a dosage of agent which is in the range of about 1 pg/kg to 4 g/kg (patient body weight), although a lower or higher dosage may be administered. The therapeutically effective dose can be lowered by using the present composition in combination with another agent. As used herein, two or more compounds are said to be administered "in combination" with each other when either (1) the physiological effects of each compound, or (2) the serum concentrations of each compound can be measured at the same time. The administration of the polyclonal antibody composition may be for either a "prophylactic" or "therapeutic" purpose. The polyclonal antibody composition is administered to the mammal in a pharmaceutically acceptable form and in a therapeutically

effective concentration. A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient patient. Such a polyclonal IgG preparation is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient.

[0054] Polyclonal antibody compositions of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby these materials, or their functional derivatives, are combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in Remington's Pharmaceutical Sciences (16th Ed., Osol, A., Ed., Mack, Easton, Pa. (1980)). In order to form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of one or more polyclonal antibody composition(s), together with a suitable amount of a carrier vehicle. Additional pharmaceutical methods may be employed to control the duration of action. Controlled release preparations may be achieved through the use of polymers to complex or adsorb one or more of the agents of the present invention. The controlled delivery may be exercised by selecting appropriate macromolecules (for example polyesters, polyamino acids, polyvinylpyrrolidone, ethylenevinylacetate, methylcellulose, carboxymethylcellulose, or protamine sulfate) and the concentration of macromolecules as well as the methods of incorporation in order to control release. Another possible method to control the duration of action by controlled release preparations is to incorporate polyclonal IgG preparations into particles of a polymeric material such as polyesters, polyamino acids, hydrogels, poly(lactic acid) or ethylene vinylacetate copolymers. Alternatively, instead of incorporating these agents into polymeric particles, it is possible to entrap these materials in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatine-microcapsules and poly(methylmethacrylate) microcapsules, respectively, or in

colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences (16^{th} Ed., Osol, A., Ed., Mack, Easton, Pa. (1980)).

[0055] The polyclonal antibody composition obtained by this method can be used for other purposes. The polyclonal antibody composition can be used directly as it is generated by the method, or can be further processed prior to its use. For example, the polyclonal antibody composition can be further fragmented, humanized, linked to another agent, etc.

[0056] In an embodiment, the antibody preparation described herein can be used in imaging techniques. In this particular embodiment, the polyclonal antibody composition can be coupled (i.e., physically linked) to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive materials include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

[0057] Alternatively, the polyclonal antibody composition can be coupled to a chemotherapeutic agent, a toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof) and/or a radioactive isotope (i.e., a radioconjugate). Exemplary toxins include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, *Sapaonaria*

officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

[0058] The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

EXAMPLE I – PREPARATION, TREATMENT AND QUANTIFICATIONS

[0059] *Culture of Human Peripheral Blood B Lymphocytes.* Blood samples were obtained from healthy individuals after informed consent, and peripheral blood mononuclear cells were prepared by density centrifugation over Ficoll-Paque (GE Healthcare Bio-Sciences Inc., Baie D'Urfé, QC, Canada). B lymphocytes were purified by negative selection using the StemSep CD19™ mixture according to the manufacturer's instructions (Stem Cell Technologies, Vancouver, BC, Canada). Purified human B lymphocytes were more than 95% $CD19^+$, as determined by flow cytometry. B lymphocytes were cultured in an incubator at 37°C and 10% CO_2 for five days in the presence of SCM-7 membrane preparations expressing CD154 (ratio: 340×10^6 CD154/25 000 B cells), as previously described (1,2), in IMDM supplemented with 10% ultra-low IgG FBS, 10 µg/ml insulin, 5.5 µg/ml transferrin, 6.7 ng/ml sodium selenite, antibiotics, (all from Invitrogen, Burlington, ON, Canada), 100 U/ml IL-4 (R&D Systems, Minneapolis, MN, USA), 50 U/ml IL-2 and 25 U/ml IL-10 (PeproTech, Rocky Hill, NJ, USA). Cell counts and viability were evaluated in triplicate by Trypan blue dye exclusion. Cultured B lymphocytes were always more than 96% $CD19^+$, and unless specified otherwise, viability was higher than 85%.

[0060] *2-Methoxyestradiol (2ME), Steroids, and Treatments.* 2ME (Sigma, St. Louis, MO, USA) was solubilized in DMSO at a stock concentration of 30 mM. B lymphocytes maintained in culture for 5, 7, 10 or 12 days were seeded at 250 000 cells/ml in the culture medium described above supplemented with 10 ng/ml IL-6 and variable concentrations of 2ME ranging from 0.25 to 0.75 µM). Control experiments without 2ME were normalized for the presence of 2ME diluent by the addition of an equivalent volume of DMSO. Cells were

treated for three days before flow cytometry analyses and viability measurements.

[0061] *Flow Cytometry Analyses.* Allophycocyanin-conjugated anti-CD19, PE-conjugated anti-CD138, allophycocyanin- and PE-conjugated isotype controls were used in double or triple staining procedures. All antibodies (Abs) were IgG1 mouse monoclonal Abs obtained from BD Biosciences (Mountain View, CA, USA). All stainings were done using 1 µg of each Ab with 1×10^6 cells at 4°C. Cells were fixed with 2% paraformaldehyde. In all analyses, more than 95% of the cells were double-negative when using isotype-matched control Abs. The proportion of $CD19^+$ or $CD138^+$ cells was determined by flow cytometry on a FACSCalibur™ apparatus (BD Biosciences). A minimum of 10 000 gated events were acquired, and analyses were done using CellQuest Pro™ and FCSExpress™ softwares (De Novo Software, Thornhill, ON, Canada).

[0062] *Purification of CD138+ cells.* B lymphocytes $CD138^+$ cells were purified by positive selection using the EasySep™ Human CD138 selection kit, according to the manufacturer's instructions (Stem Cell Technologies). Purified cells were higher than 70% $CD138^+$, as determined by flow cytometry.

[0063] *Determination of IgG and IgM Secretion Rates.* Human B lymphocytes harvested after steroid treatment or purified CD138+ cells were washed with PBS-glucose and seeded at 1 to 2×10^6 cells/ml in IMDM. Supernatants were collected after 24 hours of incubation, and IgG and IgM concentrations were determined by standard ELISA. Results were normalized as a function of the total number of viable cells, and expressed in µg Ig/10^6 cells/24 hours.

[0064] *Isoelectrofocusing.* Isoelectrofocusing (IEF) was performed on a 5% polyacrylamide gel containing pI 3-10 ampholytes. Sham-treated, steroid-treated, and purified $CD138^+$ cells obtained after treatment were washed with PBS-glucose and seeded at 1 to 2×10^6 cells/ml in IMDM. Supernatants were collected after 24 hours of incubation and were diluted in deionized water

containing 2% pI 3-10 ampholytes and loaded onto the gel. An IEF marker (Bio-Rad Laboratories, Mississauga, ON, Canada) and a control, purified human monoclonal antibody were also loaded on the gel. Following electrophoresis, proteins were transferred onto nitrocellulose membranes (Millipore Canada Ltd., Ville St-Laurent, QC, Canada) and incubated with a peroxydase-labeled goat anti-human IgG, Fc-specific polyclonal antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA). Isoelectrofocused proteins were revealed using standard electrochemiluminescent detection reagent (GE Healthcare Bio-Sciences Inc.).

[0065] *Analysis of IgG Transcript Subclasses.* Total RNA was isolated from 10^6 untreated, steroid-treated, and purified $CD138^+$ cells obtained after treatment using the Absolutely RNA™ extraction kit (Stratagene, La Jolla, CA, USA). One µg of RNA was used to prepare cDNA using M-MLV reverse transcriptase (Invitrogen) and oligo-dT. Amplification by the polymerase chain reaction (PCR) was performed using AmpliTaq Gold™ (Roche Diagnostics, Laval, QC, Canada) according to the manufacturer's instructions, and primers specific for each IgG subclass (1, 2, 3 and 4) (3). PCR products were resolved on a 1% agarose gel stained with GelRed 30™ (Biotium, Inc., Hayward, CA, USA).

EXAMPLE II – EFFECTS OF STEROID TREATMENT ON *IN VITRO*-CULTURED NORMAL B LYMPHOCYTES

[0066] A dose-response experiment was performed to measure the effect of 2ME on B lymphocytes that had been maintained in culture for 5 to 12 days. Following specified culture periods, cells were treated with 2ME at a final concentration of 0.25 µM, 0.5 µM or 0.75 µM. After three days of 2ME treatment, cells were harvested and the proliferation and viability were determined. As shown in Table 1, the addition of 0.25 µM 2ME did not significantly influence proliferation and viability. However, at final concentrations of 0.5 and 0.75 µM, 2ME decreased the proliferation of B lymphocytes in a dose-dependent manner. These results are consistent with previous reports indicating that elevated (> 1 µM) doses of 2ME inhibit cellular proliferation. The

results presented herein also suggest that 2ME altered cell viability, especially at a concentration of 0.75 µM, and to a lesser extent at 0.5 and 0.25 µM (Fig. 1). Concentrations of 2ME above 1 µM induced massive cell death, resulting in a cell viability of less than 10%.

[0067] Unexpectedly, it has been observed that 2ME induced the expression of CD138, a specific plasma cell marker (Table 1). At a concentration of 0.25 µM, 2ME did not exert any effect on CD138 expression. However, at a concentration of 0.5 µM, conversion of a significant proportion of B lymphocytes into CD138+ plasma cells was observed, but the magnitude of the effect appeared to be dependent on the time span of the B-cell culture before 2ME addition. After treatment with 0.5 µM 2ME, the proportion of $CD138^+$ cells increased from 2.5% to 7.5%, 2.5% to 9%, 5% to 12% and 5.5% to 7%, in cultures maintained for respectively 5 days, 7 days 10 days and 12 days prior to 2ME addition. 2ME treatment at a final concentration of 0.75 µM appeared to have a greater impact on the conversion of B cells into $CD138^+$ cells, but the effect was still dependent on the culture time preceding 2ME addition. The proportion of $CD138^+$ cells increased from 2.6% to 26%, 3.3% to 21.6%, 6.9% to 13.4% and 5.7% to 25.8% in cultures maintained for respectively 5 days, 7 days, 10 days and 12 days prior to 2ME addition. Collectively, these results suggest that 2ME induces the conversion of a significant proportion of normal human peripheral blood B lymphocytes into $CD138^+$ plasma cells in a dose-dependent manner.

Table 1. Effect of 2ME on the Proliferation, Viability and Conversion of Normal Human B Lymphocytes into $CD138^+$ Plasma Cells. Refer to Fig. 1 for overall experimental design. Normal human B lymphocytes were isolated from peripheral blood and grown for up to 15 days without treatment. Aliquots of this untreated culture were collected after 5 (Experiment 1), 7 (Experiment 2), 10 (Experiment 3) or 12 (Experiment 4) days of culture and treated with 0.25, 0.5 or 0.75 µM 2ME. After three days of 2ME treatment, cells were harvested for proliferation and viability assessments by counting viable cells with a haemacytometer after Trypan blue dye staining, and the percentages of $CD138^+$ cells were determined by flow cytometry. Results for treated cultures were compared to those of the untreated culture sampled on the same day. "DMSO control" refers to cultures treated with DMSO at the same final concentration as cultures treated with 2ME. Sham-treated ("Untreated") B lymphocyte cultures are also presented. n/d, not determined.

Experiment	Day of culture	Untreated			DMSO control			0.25 µM 2ME		
		Fold expansion	Viability	%CD138+	Fold expansion	Viability	%CD138+	Fold expansion	Viability	%CD138+
-	0	1	87%	< 1%	1	n/d	n/d	1	n/d	n/d
1	5	4.4	86%	n/d	n/d	n/d	n/d	n/d	n/d	n/d
	8	31.7	88%	2.6%	35.1	87%	2.0%	30.5	87%	2.5%
2	7	19.9	89%	n/d	n/d	n/d	n/d	n/d	n/d	n/d
	10	199.2	90%	3.3%	167.0	89%	2.6%	178.6	86%	2.7%
3	13	1302.8	86%	6.9%	850.0	82%	7.5%	715.0	83%	7.6%
4	12	603.8	87%	n/d	n/d	n/d	n/d	n/d	n/d	n/d
	15	5026.3	80%	5.7%	3707.1	84%	5.1%	3827.8	86%	6.1%

Table 1 (cont'd). Effect of 2ME on the Proliferation, Viability and Conversion of Normal Human B Lymphocytes into CD138+ Plasma Cells. Refer to Fig. 1 for overall experimental design. Normal human B lymphocytes were isolated from peripheral blood and grown for up to 15 days without treatment. Aliquots of this untreated culture were collected after 5 (Experiment 1), 7 (Experiment 2), 10 (Experiment 3) or 12 (Experiment 4) days of culture and treated with 0.25, 0.5 or 0.75 µM 2ME. After three days of 2ME treatment, cells were harvested for proliferation and viability assessments by counting viable cells with a haemacytometer after Trypan blue dye staining, and the percentages of CD138+ cells were determined by flow cytometry. Results for treated cultures were compared to those of the untreated culture sampled on the same day. "DMSO control" refers to cultures treated with DMSO at the same final concentration as cultures treated with 2ME. Sham-treated ("Untreated") B lymphocyte cultures are also presented. n/d, not determined.

Experiment	Day of culture	0.5 µM 2ME			0.75 µM 2ME		
		Fold expansion	viability	%CD138+	Fold expansion	viability	%CD138+
-	0	1	n/d	n/d	1	n/d	n/d
1	5	n/d	n/d	n/d	n/d	n/d	n/d
	8*	14.2	82%	7.0%	6.2	66%	26.0%
2	7	n/d	n/d	n/d	n/d	n/d	n/d
	10*	89.7	81%	8.6%	28.9	62%	21.6%
3	13*	558.1	78%	11.7%	356.2	68%	13.4%
4	12	n/d	n/d	n/d	n/d	n/d	n/d
	15	3622.6	79%	7.1%	780.9	55%	25.8%

[0068] In addition to CD138 expression, normal plasma cells are usually characterized by a high level of Ig secretion. Ig secretion by 2ME-converted $CD138^+$ cells was analyzed, relative to the secretion rates of untreated and 0.75 µM 2ME-treated B lymphocytes. As shown in Table 2, IgG and IgM secretion rates increased by 7- and 6-fold, respectively, following 2ME treatment of B lymphocytes that had been in culture for five days prior to 2ME addition. Furthermore, $CD138^+$ cells purified after treating B-cell cultures with 2ME secreted IgG at a rate of 151 µg/10^6 cells/24hours, which is 28-fold higher than untreated and unselected B lymphocytes, and close to the estimated secretion rate of plasma cells *in vivo* (~200 µg/10^6 cells/24hours). These results strongly suggest that $CD138^+$ cells obtained after treatment of human B lymphocytes with 2ME were functional plasma cells.

Table 2. Ig Secretion by B Lymphocytes in Response to 2ME and by CD138-Enriched Plasma Cells Derived from 2ME Treatment of B Lymphocytes. B lymphocytes cultured for five days were split into equal aliquots of 2 mL at a final concentration of 250 000 cells/mL and were either maintained in culture for an additional three days without treatment, or treated for three days with 0.75 µM 2ME, in 6 wells plates. CD138+ cells were purified from the 2ME-treated culture. Secreted IgG and IgM concentrations were determine by ELISA, as described in Materials and Methods, for untreated B lymphocytes, 2ME-treated B lymphocytes, and purified CD138+ cells derived from treatment of B lymphocytes with 2ME. Results are expressed in µg Ig/10^6 cells/24 hours, and represent means +/- standard deviations of three independent experiments.

Treatment	**IgG**		**IgM**	
	µg /10^6 cells/24 hours	Fold incr.	µg /10^6 cells/24 hours	Fold incr.
Untreated	5.42 ± 0.13	1X	0.4 ± 0.005	1X
2ME-Treated	35.9 ± 0.89	7X	2.24 ± 0.16	6X
2ME-Treated, $CD138^+$-purified	151 ± 3.87	28X	4.66 ± 0.47	10X

[0069] The polyclonality of Igs secreted by 2ME-induced plasma cells was investigated by isoelectrofucusing (IEF). As shown in Fig. 2, a smear signal, typical of polyclonal Ig preparations, could be observed in culture supernatants from untreated and 2ME-treated B lymphocytes, as well as purified $CD138^+$ cells obtained after 2ME treatment of B cells. These results suggest that the polyclonality of secreted Igs was not biased by 2ME treatment. Transcripts encoding the four IgG subclasses were then examined by reverse transcriptase-PCR (RT-PCR) in RNA samples from untreated and 2ME-treated B lymphocytes as well as CD138-purified cells obtained after 2ME treatment of B cells. As shown in Fig. 3, transcripts from all four IgG subclasses (IgG_1, IgG_2, IgG_3 and IgG_4) were detected, independently of 2ME treatment, and in CD138-purified cells. Furthermore, measurements of the four IgG subclass concentrations in culture supernatants by ELISA confirmed that 2ME treatment did not induce bias in IgG subclass secretion (Table 3). These results indicate that 2ME treatment does not induce a bias in the repertoire of secreted Igs.

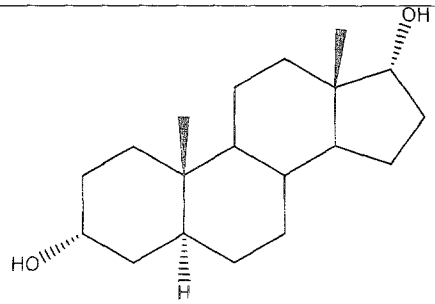
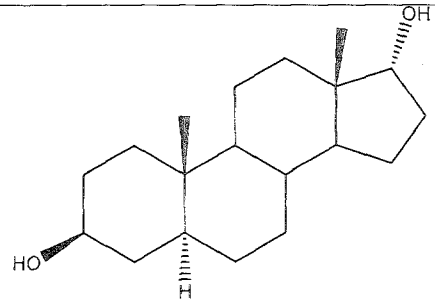
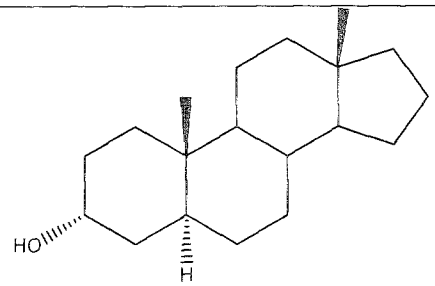
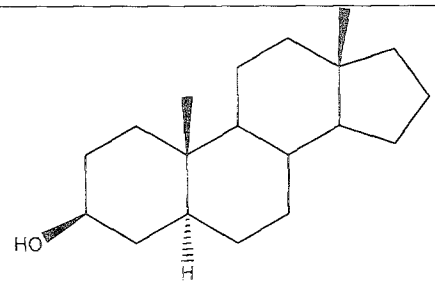
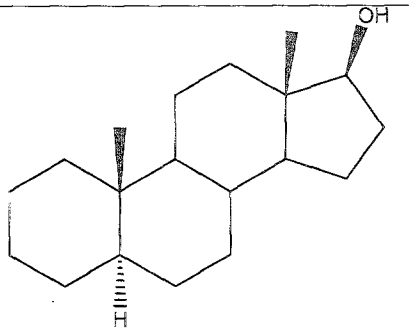
Table 3. B lymphocytes cultured for five days were either maintained untreated for an additional three days or treated for three days with 0.75 µM 2ME. An aliquot of 2ME-treated B lymphocytes was also used for purification of $CD138^+$ cells. Secreted IgG concentrations were determined by ELISA for each subclass for untreated B lymphocytes, 2ME-treated B lymphocytes, and CD138+ cells purified from 2ME-treated B lymphocytes. Results are expressed in µg IgG/10^6 cells/24 hours, and represent means +/- standard deviations of three independent experiments.

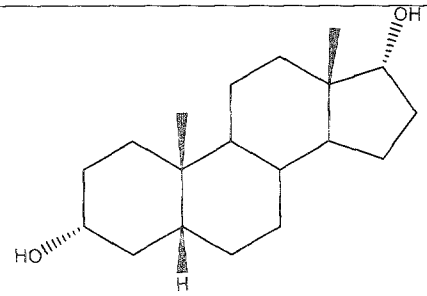
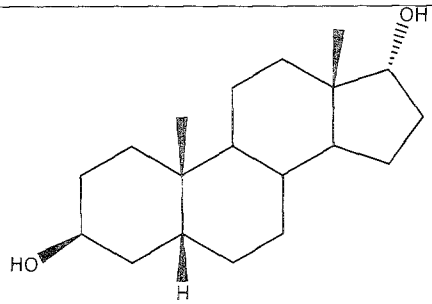
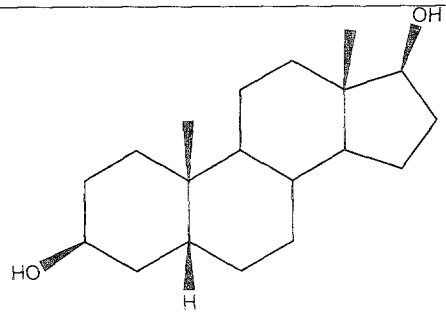
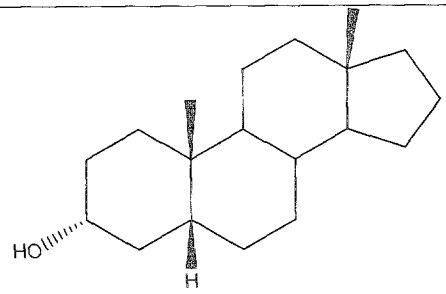
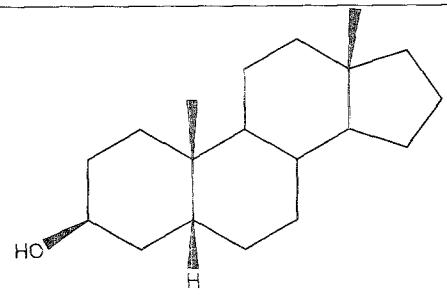
Treatment	IgG_1			IgG_2			IgG_3			IgG_4		
	µg/10^6 cells/ 24 h	Fold increase	% of total IgG	µg/ 10^6 cells/ 24 h	Fold increase	% of total IgG	µg/ 10^6 cells/ 24 h	Fold increase	% of total IgG	µg/ 10^6 cells/ 24 h	Fold increase	% of total IgG
Untreated	4 ± 0.12	1X	62%	1.78 ± 0.15	1X	28%	0.261 ± 0.021	1X	4%	0.377 ± 0.03	1X	6%
2ME-Treated	27.7 ± 0.5	7X	60%	15.7 ± 0.65	9X	33.8%	2.21 ± 0.14	8X	4.7%	0.727 ± 0.008	2X	1.5%
2ME-Treated $CD138^+$-purified	107 ± 4.1	27X	48%	97.8 ± 4.8	54X	44%	11.4 ± 0.6	44X	5%	7.91 ± 0.23	21X	3%

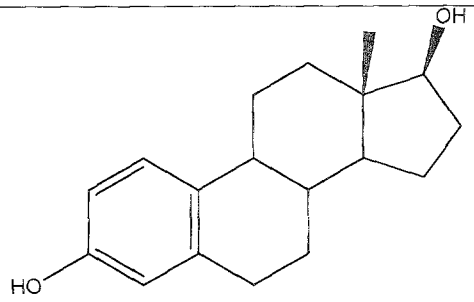
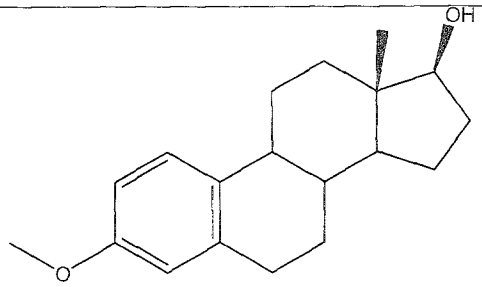
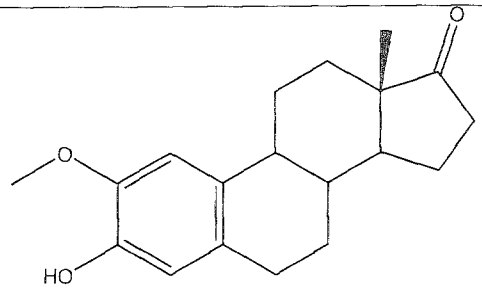
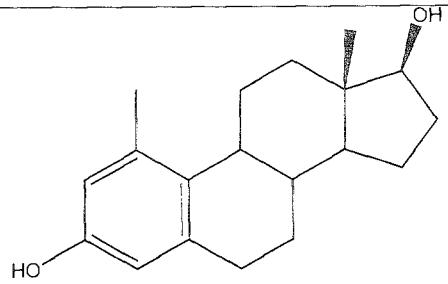
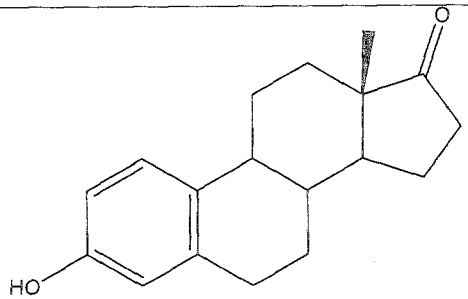
[0070] To further extend the characterization of 2ME-induced plasma cells derived from human peripheral blood B lymphocytes, analysis of the evolution of CD19, CD27, CD38, CD138 and surface IgG by FACS in either single or double stainings was performed. No significant variations in CD19 expression could be detected between 2ME-treated (90.6%) and untreated (91.3%) cells. In contrast, 2ME markedly reduced surface IgG expression (sIgG). Indeed, 19% of untreated cells expressed sIgG, whereas only 2% of cells remained sIgG+ following 2ME treatment. Unexpectedly, it was observed that an increase in the CD27+ cell population following 2ME treatment, from 60% in untreated cells to 91% in treated cells (Fig. 3). This observation could suggest that 2ME-induced alterations in cell viability preferentially affect naïve B cells (CD27−). Additionally, it was further shown that most of the 2ME-converted CD138+ cells are also CD27+, suggesting that 2ME preferentially induces the conversion of memory B lymphocytes into plasma cells. It was also noted that two distinct populations of CD38+ cells (CD38lo and CD38hi) could be detected in both untreated and 2ME-treated B lymphocytes. While no variations in the percentage of total CD38+ cells were observed after 2ME treatment, the relative proportions of these two CD38+ populations were substantially modified after treatment. The percentage of CD38lo decreased from 51% to 25% after 2ME treatment, whereas the proportion of CD38hi increased from 35% to 65% after treatment.

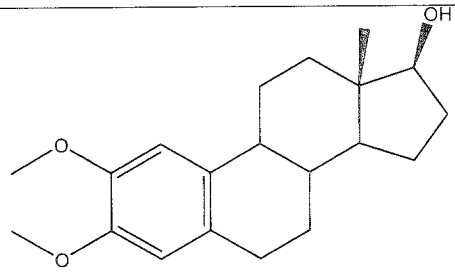
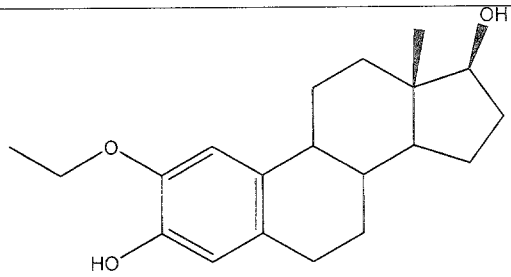
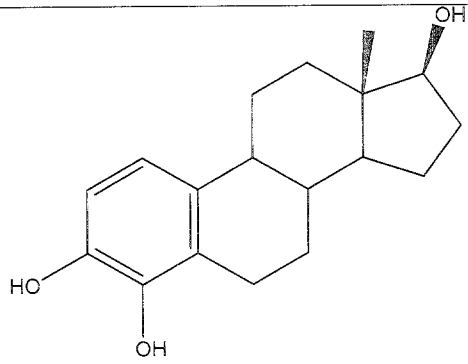
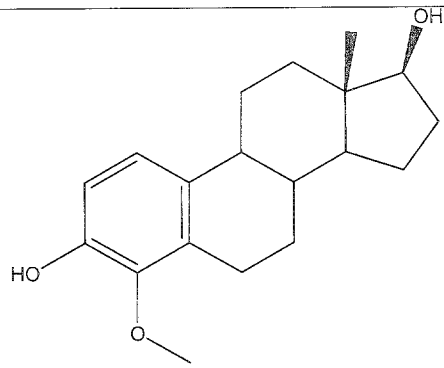
[0071] The effect of other steroidal compounds on the IgG secretion rate of normal human B lymphocytes has also been analyzed. The compounds, shown in Table 4 (including members of various steroid families such as androstanes, estranes and pregnanes) were obtained from a commercial source (Steraloids Inc., Newport, RI, USA) and used without further purification.

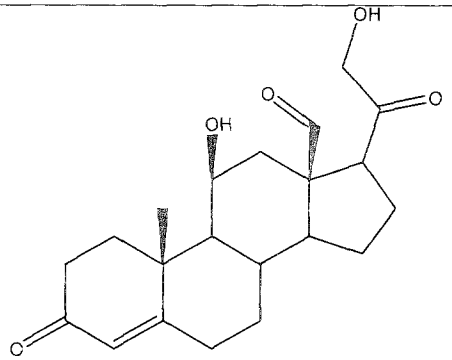
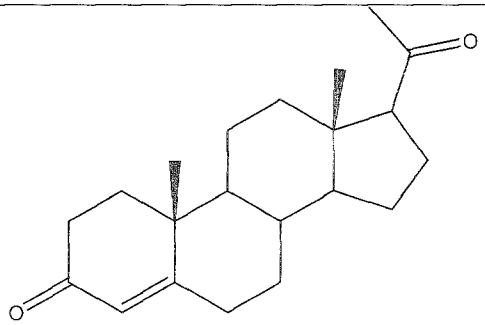
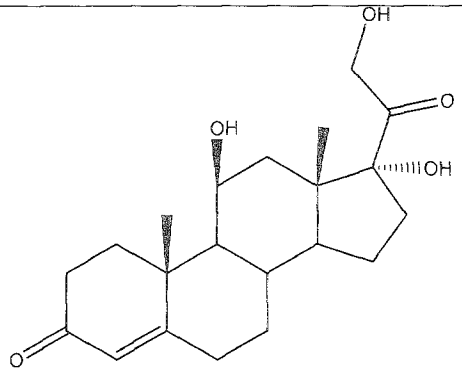
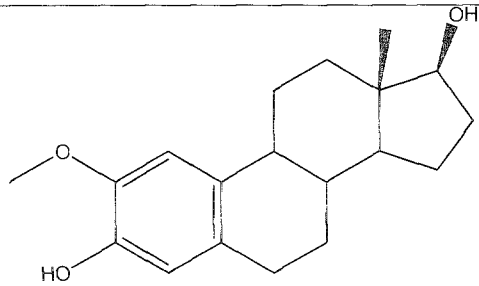
Table 4. Details on compounds used in this example.

COMPOUND	STRUCTURE	NAME
2		5α-androstan-3α,17α-diol
3		5α-androstan-3β,17α-diol
4		5α-androstan-3α-ol
5		5α-androstan-3β-ol
6		5α-androstan-17β-ol

COMPOUND	STRUCTURE	NAME
7		5β-androstan-3α,17α-diol
8		5β-androstan-3β,17α-diol
9		5β-androstan-3β,17β-diol
10		5β-androstan-3α-ol
11		5β-androstan-3β-ol

COMPOUND	STRUCTURE	NAME
12		17β-estradiol (E2)
13		17β-estradiol 3-methyl ether
14		2-methoxyestrone
15		1-methylestradiol
16		estrone (E1) 3-hydroxy-1,2,5(10)estratriene -17one

COMPOUND	STRUCTURE	NAME
17		2-methoxyestradiol 3-methyl ether
18		2-ethoxyestradiol
20		4-hydroxyestradiol
21		4-methoxyestradiol

COMPOUND	STRUCTURE	NAME
22		aldosterone
23		progesterone
24		cortisol
19 (2ME)		2-methoxyestradiol

[0072] The compounds were solubilized in DMSO at a stock concentration of 30 mM and added to cultured B lymphocytes at a final concentration of 0.75 µM. After three days of exposure, cell viability was determined, culture supernatants were harvested and their IgG content were determined by ELISA. As shown in

Fig. 4, B-lymphocyte viability was less affected by the different steroids compared to 2ME, with the exception of 2-ethoxyestradiol. Furthermore, at the concentration used (0.75 µM), most of the compounds stimulated IgG secretion by B lymphocytes, however to a lesser extent than 2ME used at the same concentration.

[0073] Further characterization of the effects of these steroidal compounds on the conversion of B cells into $CD138^+$ cells has been made. Briefly, $CD19^+$ purified human peripheral blood B lymphocytes were cultured in the presence of 100 U/ml IL-4, 50 U/ml IL-2, 25 U/ml IL-10 and SCM-7 membrane preparations expressing CD154 (ratio: 340 x 10^6 CD154/25 000 B cells), as described above. After four days of culture, cells were split into equal aliquots of 200 µl at a final concentration of 250 000 cells/ml and cultured in 96 wells plate for an additional three days in the same culture medium, either without any additive (untreated), with DMSO or with the indicated concentrations of steroids in DMSO. At the end of the incubation, cells were harvested, and analyzed for CD138 expression by flow cytometry. Results are shown in Table 5. Cell viability is presented in as the percentage of gated cells. Results represent means +/- standard deviations (for untreated and DMSO n = 16, for 2-Methoxyestradiol n = 9 and for other steroids n = 3).

Table 5: Effect of steroidal compounds on the viability and conversion of Normal human B lymphocytes into $CD138^+$ plasma cells.

Steroidal compound	% $CD138^+$ cells	% of gated cells
Untreated	8.6 ± 2.9	48.4 ± 10.7
DMSO	8 ± 2.8	47.1 ± 10.5
2-Methoxyestradiol (0.75 µM)	26 ± 6.5	16.6 ± 6.5
5α-Androstan-3α,17α-diol (50 µM)	16 ± 4.2	15 ± 0.9
5α-Androstan-3β,17α-diol (50 µM)	8.8 ± 1.1	25.6 ± 1.1
5α-Androstan-3α-ol (50 µM)	27.2 ± 1.6	19.5 ± 0.3
3β-Hydroxy-5α-Androstane (50 µM)	19.7 ± 1.6	4.5 ± 0.3
17β-Hydroxy-5α-Androstane (0.01 µM)	12.9 ± 1.2	27.2 ± 2.4
Etiocholan-3α,17α-diol (50 µM)	14.8 ± 1.5	9.5 ± 0.2
Etiocholan-3β,17α-diol (50 µM)	34.6 ± 4.5	11.9 ± 1.6
3β,17β-Dihydroxyetiocholane (50 µM)	33 ± 2.3	10.6 ± 0.9
Etiocholan-3α-ol (50 µM)	33.3 ± 6	3.1 ± 0.3

Steroidal compound	% $CD138^+$ cells	% of gated cells
5β-Androstan-3β-ol (10 μM)	19. ± 0.9	13 ± 2.2
E2 17β-Estradiol (50 μM)	14.5 ± 2.2	12.8 ± 4.2
17β-Estradiol 3-Methylether (50 μM)	23.9 ± 2.7	8.9 ± 0.7
2-Methoxyestrone (50 μM)	24.3 ± 0.4	10.3 ± 1.1
1-Methylestradiol (50 μM)	11.7 ± 0.8	20.4 ± 3.2
E1 Estrone 3-Hydroxy-1,3,5(10)-Estratrien-17-one (0.01 μM)	8.7 ± 1.1	55.2 ± 0.3
2-Methoxyestradiol3-Methylether (50 μM)	13.5 ± 1.6	10.8 ± 3.1
2-Ethoxyestradiol (0.5 μM)	38.8 ± 0.6	8.6 ± 0.4
4-Hydroxyestradiol (10 μM)	10.6 ± 0.2	21.6 ± 2.9
4-Methoxyestradiol (50μM)	18.5 ± 0.9	7.9 ± 06
Aldosterone (1 μM)	3.8 ± 0.4	69 ± 1.9
Progesterone (50 μM)	14.8 ± 0.6	41.3 ± 2.2
Hydrocortisone (5 μM)	4.1 ± 0.1	65.5 ± 0.2

[0074] In addition, in order to determine the effect of 5α-androstan-3α-ol on Ig secretion, B lymphocytes cultured for five days were split into equal aliquots of 2 mL at a final concentration of 250 000 cells/mL and were either maintained in culture for an additional three days without treatment, or treated for three days with 0.25 μM 5α-Androstan-3α-ol. $CD138^+$ cells were purified from the 5α-Androstan-3α-ol -treated culture, in 6 wells plates. Secreted IgG and IgM concentrations were determine by ELISA, as described in Example I, for untreated B lymphocytes, 5α-Androstan-3α-ol-treated B lymphocytes, and purified $CD138^+$ cells derived from treatment of B lymphocytes with 5α-Androstan-3α-ol. Results, presented in Table 6, are expressed in μg Ig/10^6 cells/24 hours, and represent means +/- standard deviations of three independent experiments.

Table 6. Ig secretion by B lymphocytes in response to 5α-Androstan-3α-ol and by CD138-enriched plasma cells derived from 5α-Androstan-3α-ol-treatment of B lymphocytes.

Conditions	IgG		IgM	
	µg IgG/10^6 cells/24 hours	Fold increase	µg IgM/10^6 cells/24 hours	Fold increase
Untreated	29.1 ± 2.5	1X	1.3 ± 0.2	1X
Treated 5α-Androstan-3α-ol	37.5 ± 4.7	1.3X +	1.1 ± 0.1	0.9X -
Treated 2-ME $CD138^+$ purified	128.8 ± 22.1	4.4X +	1.4 ±	1.1X +

[0075] B lymphocytes cultured for five days were split into equal aliquots of 1 mL at a final concentration of 250 000 cells3mL and were either maintained untreated for an additional three days or treated for three days with 0.25 µM 5α-Androstan-3α-ol, in 6 wells plates. An aliquot of 5α-Androstan-3α-ol -treated B lymphocytes was also used for purification of $CD138^+$ cells. Secreted IgG concentrations were determined by ELISA for each subclass for untreated B lymphocytes, 5α-Androstan-3α-ol-treated B lymphocytes, and $CD138^+$ cells purified from 5α-Androstan-3α-ol-treated B lymphocytes. Results are presented in Table 7 and are expressed in µg IgG/10^6 cells/24 hours, and represent means +/- standard deviations of three independent experiments.

Table 7. IgG subclass distribution of antibodies produced by cells treated with 5α-Androstan-3α-ol.

Conditions	IgG1			IgG2			IgG3			IgG4		
	µg IgG/10^6 cells/24 h	Fold increase	% of total IgG	µg IgG/10^6 cells/24 h	Fold increase	% of total IgG	µg IgG/10^6 cells/24 h	Fold increase	% of total IgG	µg IgG/10^6 cells/24 h	Fold increase	% of total IgG
Untreated	18.8 ±4.8	1X	45%	9.7 ±1.8	1X	24%	1.1±0.1	1X	3%	11.7 ±0.22	1X	28%
Treated	23.0 ±4.1	1,2X	49%	11.7 ±1.0	1,2X	25%	1.0 ±0.2	0,9X	2%	11.3±3.8	1X	24%
Treated and $CD138^+$ purified	58.0 ±3.1	3,1X	60%	28.3 ±1.2	3X	29%	2.7 ±0.4	2,5X	3%	7.4 ±0.8	0,6X	8%

[0076] Cell supernatants used for the ELISA results of Table 7 were subjected to isoelectrofocusing, as described above. Supernatants from untreated B lymphocytes (ut), B lymphocytes treated with 0.25 µM 5α-Androstan-3α-ol and $CD138^+$ cells purified from 5α-Androstan-3α-ol-treated B lymphocytes (CD138) were analyzed. A control corresponding to a monoclonal human antibody (ct), and an IEF marker were run in parallel.

[0077] Without wishing to be bound to theory, it is respectfully suggested that 2ME induces the conversion of normal human B lymphocytes into plasma cells without altering their Ig repertoire. Moreover, some members of the androstane, estrane and pregnane families also appear capable of converting B cells into $CD138^+$ plasma cells and/or increasing IgG secretion by B lymphocytes. Normal human B lymphocytes cultured *in vitro* could thus be a valuable source of Igs for the treatment of immune-related disorders. Mass-scale quantities of normal human Igs could be produced as a substitute for plasma-derived IVIg preparations. The new method described herein could thus alleviate potential shortages in IVIg.

EXAMPLE III – EFFECT OF PHORBOL-12-MYRISTATE-13-ACETATE ON NORMAL HUMAN B LYMPHOCYTES AND MULTIPLE MYELOMA CELL LINES

[0078] It has been previously reported that 2ME slightly increases the secretion of aberrant Ig light chains by multiple myeloma cell lines, which are derived from neoplasms of plasma cell origin characterized by the ubiquitous expression of the CD138 plasma cell marker (4,5,6). Further, as shown herein, it has been observed that phorbol-12-myristate-13-acetate (PMA) also slightly increases the secretion of Ig light chains by a multiple myeloma cell line (RPMI), as does 2ME (Table 8). It was thus investigated whether PMA could also enhance IgG secretion by normal human B lymphocytes. As shown in Table 5, PMA has no effect on IgG or IgM secretion by normal human B lymphocytes. In addition, flow cytometry analyses revealed that no conversion of B lymphocytes into $CD138^+$ cells occurred in response to PMA. These results show that the responses of multiple myeloma cell lines to 2ME and PMA are similar, whereas

the responses of normal human B lymphocytes to these two agents are clearly distinct. This finding underlines that there are important differences in the biology and the physiology of multiple myeloma cells relative to normal human B lymphocytes.

[0079] Table 8. Ig secretion by B lymphocytes and RPMI cells in response to 2ME or PMA treatment. B lymphocytes and RPMI cells were cultured for five days and either maintained in culture for an additional three days without additional agent or treated for three days with 0.75 µM 2ME or 50 nM PMA. Secreted IgG (normal B cells) and λ chain (RPMI cells) concentrations were determine by ELISA, as described in Example I. Results are expressed in µg Ig/10^6 cells/24 hours, and represent means +/- standard deviations of three independent experiments.

Treatment	RPMI µg λ chain/10^6 cells/24 hours	B lymphocytes µg IgG/10^6 cells/24 hours
Untreated	2 ± 0.36	5.42 ± 0.13
0,75 µM 2ME, 72h	2.23 ± 1.25	35.9 ± 0.89
50 nM PMA, 72h	2.58 ± 1.71	2.02 ± 0.86

References

1. Jung, D., Neron, S., Drouin, M., and Jacques, A. (2005) J Immunol Methods 304, 78-87

2. Cayer, M. P., Drouin, M., Sea, S. P., Forest, A., Cote, S., Simard, C., Boyer, L., Jacques, A., Pineault, N., and Jung, D. (2007) J Immunol Methods 322, 118-127

3. Barbas, C. F., 3rd, Burton, D., Scott, J., and Silverman, G. J. (2001) in Phage Display : A Laboratory Manual (C. S. H. L. Press, ed), Cold Spring Harbor Laboratory Press, New York

4. Hou, J., Xiong, H., Gao, W., and Jiang, H. (2005) Leuk Res 29, 1059-1067

5. Zhou, X., Jiang, H., and Hou, J. (2007) Leuk Res 31, 1259-1265

6. Jiang, H., Gao, W.-R., Man-Yuen Sze, D., Xiong, H., et Hou, J. (2007) Int J Hematol 86, 429-437

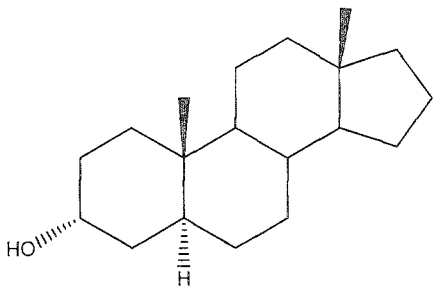
[0080] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS

1. A method of inducing the conversion of a normal B lymphocyte into a plasma cell, said method comprising exposing the normal B lymphocyte to a steroidal compound, thereby favoring the conversion of the normal B lymphocyte into a plasma cell.

2. The method of claim 1, wherein the steroidal compound has the formula of any one of compounds 2, 4, 5, 8, 9, 10, 11, 12, 13, 14, 18, 19, 21 and 23 as set forth in Table 4.

3. The method of claim 1, wherein the steroidal compound has the formula:



4. The method of any one of claims 1 to 3, wherein the exposure occurs *in vitro*.

5. The method of claim 4, wherein the steroidal compound is added to a culture medium for the culture of the normal B lymphocyte.

6. The method of any one of claims 1 to 5, further comprising exposing an initial population of B lymphocytes comprising the normal B lymphocyte to the steroidal compound to provide a cultured population of B lymphocytes.

7. The method of claim 6, wherein the initial population of B lymphocytes comprises CD19+ cells.

8. The method of claim 6 or 7, wherein the initial population of B lymphocytes comprises CD27+ cells.

9. The method of any one of claims 6 to 8, wherein B lymphocytes of the initial population are normal B lymphocytes.

10. The method of claim 7, wherein the percentage of CD19+ cells in the initial population of B lymphocytes is higher than about 90%.

11. The method of claim 7, wherein the percentage of CD19+ cells in the initial population of B lymphocytes is higher than about 95%.

12. The method of any one of claims 6 to 11, wherein the cultured population of B lymphocytes comprises CD138+ cells.

13. The method of claim 12, wherein the percentage of CD138+ cells in the cultured population of B lymphocytes is at least about 10.0%.

14. The method of claim 13, wherein the percentage of CD138+ cells in the cultured population of B lymphocytes is at least about 15.0%.

15. The method of claim 14, wherein the percentage of CD138+ cells in the cultured population of B lymphocytes is at least about 20.0%.

16. The method of claim 15, wherein the percentage of CD138+ cells in the cultured population of B lymphocytes is at least about 25.0%.

17. The method of claim 16, wherein the percentage of CD138+ cells in the cultured population of B lymphocytes is at least about 30.0%.

18. The method of claim 17, wherein the percentage of CD138+ cells in the cultured population of B lymphocytes is at least about 35.0%.

19. The method of claim 18, wherein the percentage of CD138+ cells in the cultured population of B lymphocytes is at least about 40.0%.

20. The method of any one of claims 6 to 16, further comprising isolating CD138+ cells from the cultured population of B lymphocytes.

21. The method of claim 17, further comprising culturing the isolated CD138+ cells.

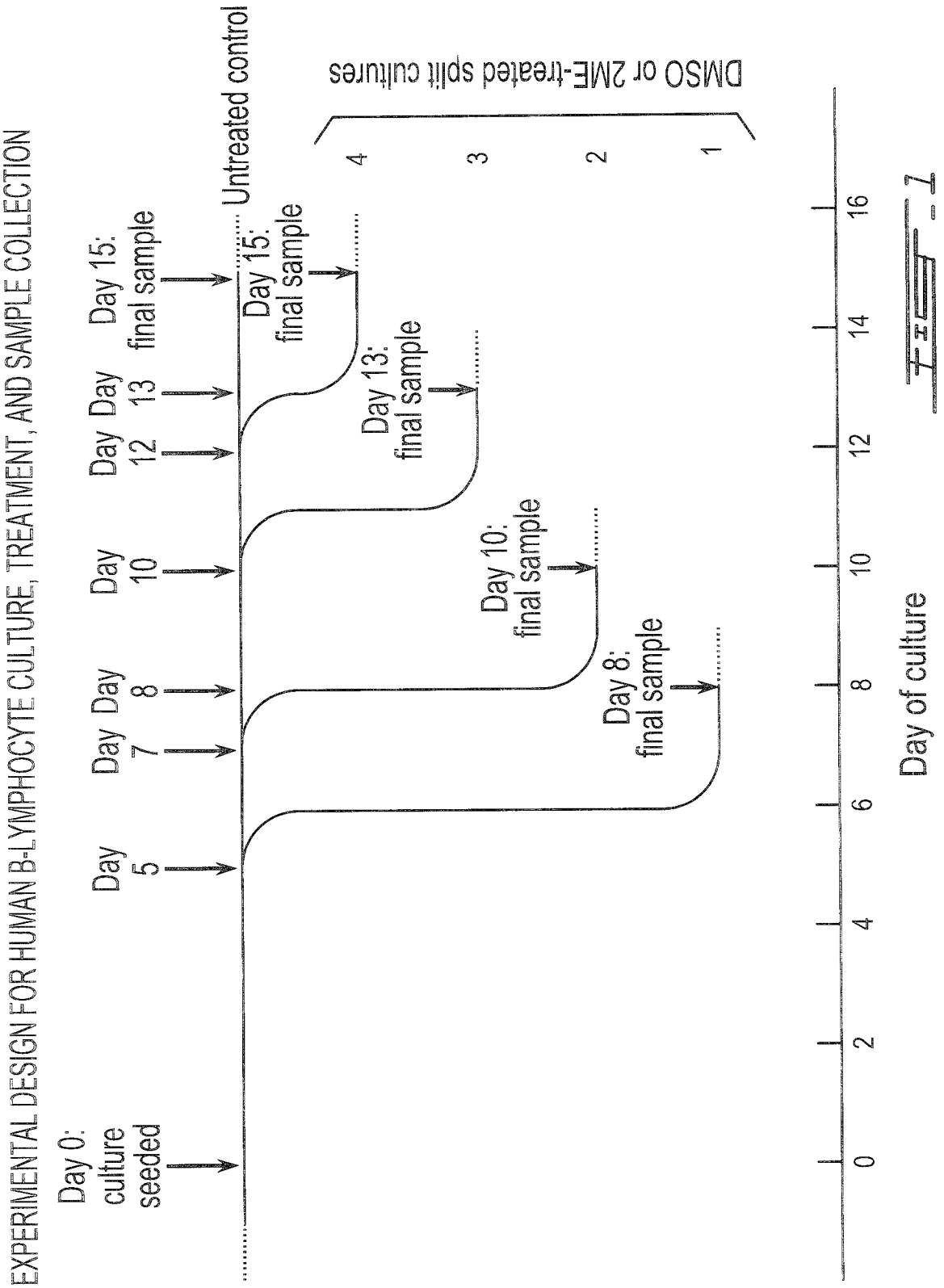

EXPERIMENTAL DESIGN FOR HUMAN B-LYMPHOCYTE CULTURE, TREATMENT, AND SAMPLE COLLECTION
Day 0: culture seeded
Day 5
Day 7
Day 8
Day 10
Day 12
Day 13
Day 15: final sample
Untreated control
Day 15: final sample
DMSO or 2ME-treated split cultures
4
3
2
1
Day 13: final sample
Day 10: final sample
Day 8: final sample
0
2
4
6
8
10
12
14
16
Day of culture

Fig. 1

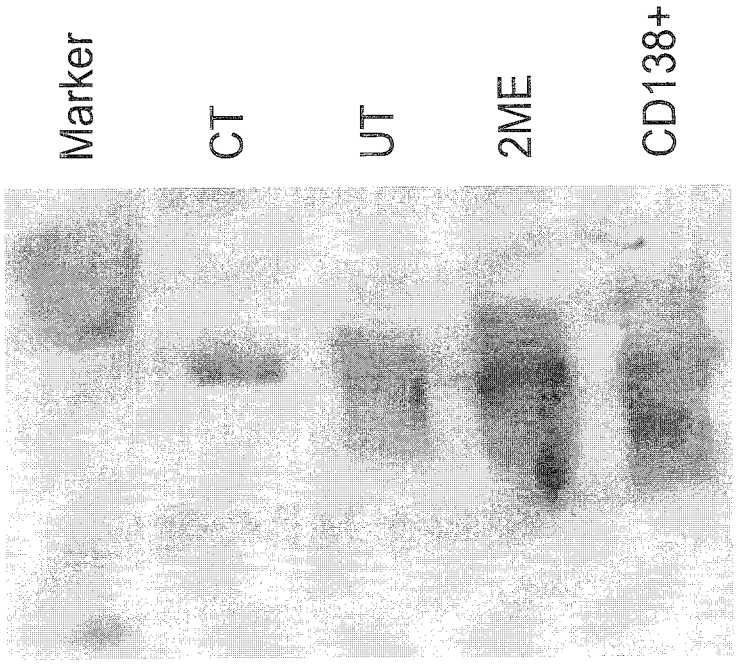


Fig. 2

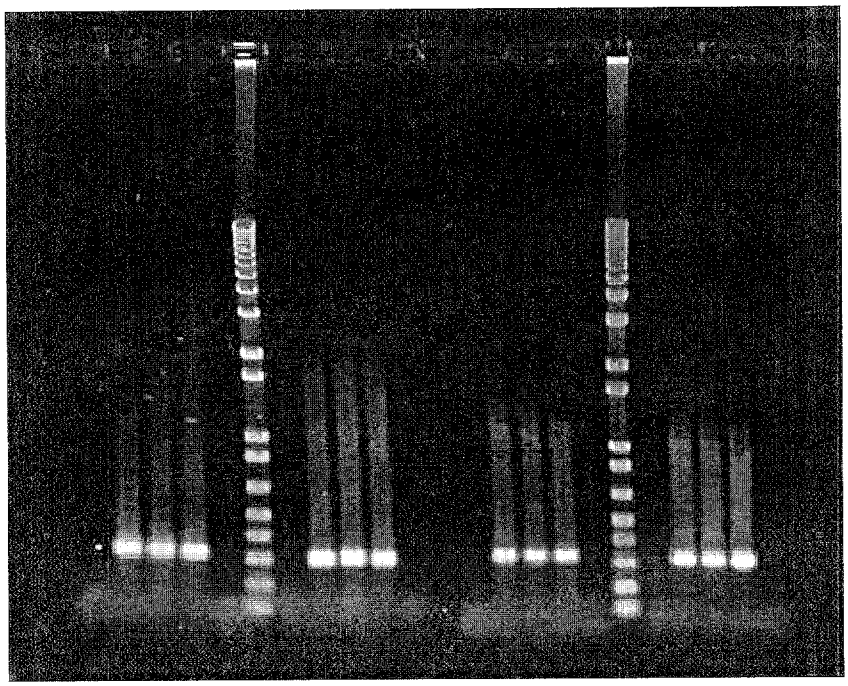


Fig. 3

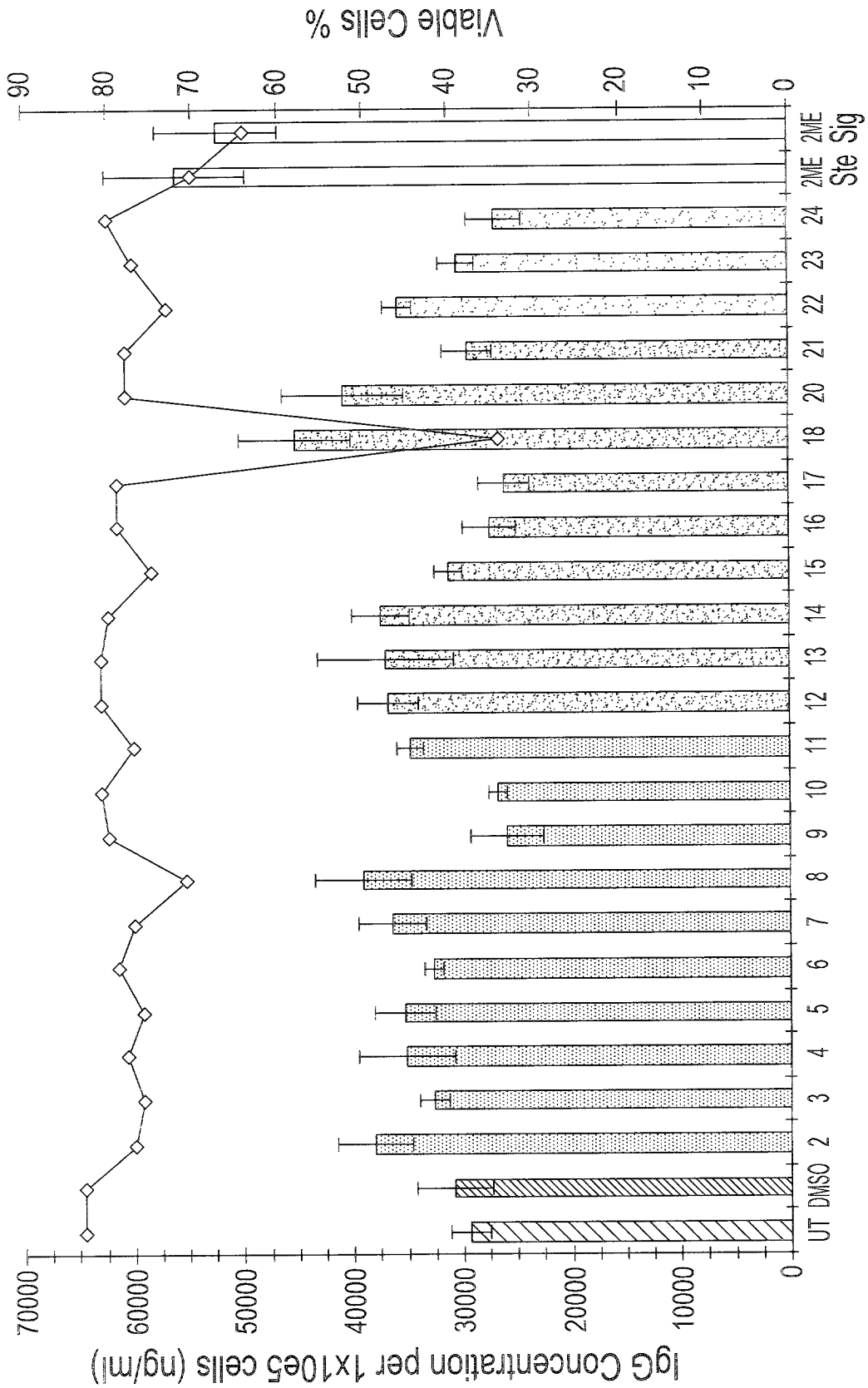

IgG Concentration per 1x10e5 cells (ng/ml)
70000
60000
50000
40000
30000
20000
10000
0
Viable Cells %
90
80
70
60
50
40
30
20
10
0
UT DMSO 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 20 21 22 23 24 2ME 2ME
Ste Sig

Fig - 4

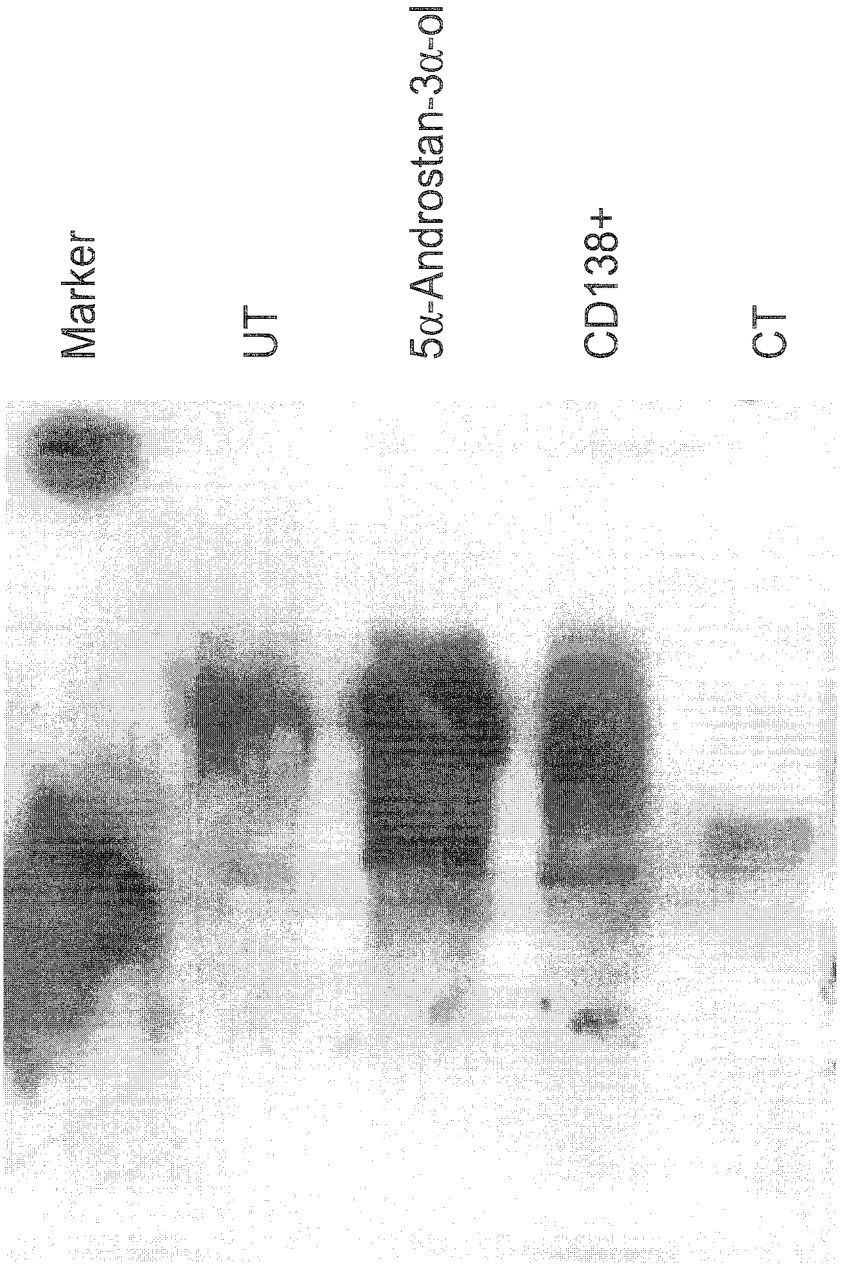


Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2010/001060

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C12N 5/078*** (2010.01) , ***C12N 5/0781*** (2010.01) , *A61K 31/565* (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)

IPC: ***C12N 5/078*** (2010.01) , ***C12N 5/0781*** (2010.01) , *A61K 31/565* (2006.01), *C12N5/06 B11B* (ECLA)

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

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

Canadian Patent Database, EPOQUE-Epodoc, TotalPatent, PubMed, CAPlus, BIOSIS and Scopus; Search terms: steroid, B cells, plasma cells, in vitro, cell culture, antibody, immunoglobulin, androstan, 2-methoxyestradiol (2-ME), estradiol, estrone, progesterone, cortisol, aldosterone, etiocholan, corticosteroid, CD138, conversion and differentiation.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	CAYER, M.-P. ET AL.: "2-Methoxyestradiol induce the conversion of human peripheral blood memory B lymphocytes into plasma cells" J. IMMUNOL. METHODS, 15 April 2010 (15-04-2010) vol. 355, pages 29-39, ISSN:0022-1759 see the whole document	1, 2 and 4-21
X --- Y	KALMAN, B. ET AL.: "Estradiol potentiates poke-weed mitogen-induced B cell stimulation in multiple sclerosis and healthy subjects" ACTA NEUROLOGICA SCANDINAVICA., 1989 vol. 79, pages 340-346, ISSN:0001-6314 see the whole document, especially the abstract	1, 2 and 4-19 --- 20 and 21
X --- Y	KORCZAK-KOWALSKA, G. ET AL.: "Glucosteroid (GS)-dependent immunomodulation of immunoglobulin biosynthesis *in vitro*. I. GS stimulate spontaneous immunoglobulin synthesis" ARCHIVUM IMMUNOLOGIAE ET THERAPIAE EXPERIMENTALIS, 1987 vol. 35, pages 631-636, ISSN:0004-069X see the whole document	1 and 4-19 --- 20 and 21

[X] Further documents are listed in the continuation of Box C. [X] 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 application or patent 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
27 September 2010 (27-09-2010)	29 October 2010 (29-10-2010)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Authorized officer Sandra Hurley (819) 934-7934

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2010/001060

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

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

1. [X] Claim Nos. : 1-3 and 6-21

because they relate to subject matter not required to be searched by this Authority, namely :

Although claims 1-3 and 6-21 of the present application encompass a method of medical treatment which this Authority is not obliged to search under Rule 39.1(iv) of the PCT, the search has been carried out on the basis of the alleged inventive effects of the steroidal compounds specified therein.

2. [ ] Claim Nos. :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

The concept of independent claim 1 is a method for inducing the conversion of a normal B lymphocyte into a plasma cell wherein said lymphocyte is exposed to a steroidal compound. An *a posteriori* analysis has concluded that said concept was known before the priority date of the instant application (please see the Kalman et al. and Korczak-Kowalska et al. documents cited herein). Therefore, the claims are directed to a plurality of alleged inventive concepts, wherein each of the 14 steroidal compounds specified in claim 2 represents a separate alleged invention.

Rule 13 of the PCT requires that the claims be limited to one inventive concept.

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

2. [X] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

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

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2005/052139 A2 (LEONARD, W. ET AL.) 09 June 2005 (09-06-2005) see especially abstract, page 2, line 25-page 3, line 22 and claims	20 and 21
A	ARPIN, C. ET AL.: "Generation of memory B cells and plasma cells *in vitro*" SCIENCE, 05 May 1995 (05-05-1995) vol. 268, pages 720-722, ISSN:0193-4511 cited in the application see the whole document	1-21

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2010/001060

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2005052139A2	09 June 2005 (09-06-2005)	AT474915T	15 August 2010 (15-08-2010)
		DE602004028280D1	02 September 2010 (02-09-2010)
		EP1692276A2	23 August 2006 (23-08-2006)
		EP1692276B1	21 July 2010 (21-07-2010)
		GB0609952D0	28 June 2006 (28-06-2006)
		GB2422845A	09 August 2006 (09-08-2006)
		GB2422845B	01 August 2007 (01-08-2007)
		US2006057123A1	16 March 2006 (16-03-2006)
		US7378276B2	27 May 2008 (27-05-2008)
		US2007003515A1	04 January 2007 (04-01-2007)
		US2008305076A1	11 December 2008 (11-12-2008)
		WO2005052139A3	22 September 2005 (22-09-2005)