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(71) Applicant *(for all designated States except US)*: **BAYER CORPORATION** [US/US]; 800 Dwight Way, P. O. Box 1986, Berkeley, CA 94701 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **CASSELL, Delanie** [US/US]; 6 Hampton Avenue, San Anselmo, CA 94960 (US). **TEPPER, Jeffrey, S.** [US/US]; 197 Glasgow Lane, San Carlos, CA 94070 (US). **SAMUELS, Isa** [US/US]; 4054 Oakmore Road, Oakland, CA 94602 (US). **DUBOIS-STRINGFELLOW, Nathalie** [FR/US]; 1008 The Alameda, Berkeley, CA 94707 (US).

(74) Agent: **MAHONEY, John, M.**; Bayer Corporation, 800 Dwight Way, P. O. Box 1986, Berkeley, CA 94701 (US).

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(54) **Title:** USE OF *PARAPOX* B2L PROTEIN TO TREAT CANCER AND MODIFY IMMUNE RESPONSES

(57) **Abstract:** A purified *Parapoxvirus ovis* envelope protein termed "B2L" can be used as a monotherapeutic agent. B2L protein also can be used in screening methods to identify potential therapeutic agents for modulating a subject's immune response to the B2L protein.

USE OF *PARAPOX* B2L PROTEIN TO TREAT CANCER AND MODIFY IMMUNE RESPONSES

[01] This application claims the benefit of and incorporates by reference co-pending provisional application Serial No. 60/336,694 filed December 7, 2001.

FIELD OF THE INVENTION

[02] The invention relates to the field of cancer therapy and immunotherapy. More particularly, the invention relates to the use of a purified B2L viral envelope protein of a *Parapox* virus as a therapeutic agent. The invention also relates to screening methods to identify potential therapeutic agents that modify the effect of B2L protein.

BACKGROUND OF THE INVENTION

[03] Attenuated *Parapox* viruses can be used to induce P*arapox*-specific immunity. U.S. Patent 6,162,600. In addition, the highly attenuated strain D1701 (Baypamun HK®) is used as a non-specific immunomodulator (Buttner *et al.*, *Immunol. Microbiol. Infect. Dis. 16*, 1-10, 1993) to promote immunity to heterologous pathogens. The virus also infects humans and induces nonspecific production of immune modulating cytokines in cultures of human PBMC (Buttner *et al.*, *Vet. Immunol. Immunopathol. 46*, 237-50, 1995).

[04] Attenuation of *Parapoxvirus ovis*, however, is time-consuming, taking from 100 to 200 culture passages; according to WO 95/22978, it takes from three to five years to perform each 100 passages, depending on the species of virus used. Attenuation can, therefore, "encompass a period lasting from ten to twenty years." (See WO 95/22978, page 9).

[05] WO 95/22978 discloses the use of combinations of two or more individual *Parapox* virus components as "multipotent paramunity inducers" for use as adjuvant therapy for tumors and the prevention of metastases. The components can be individual polypeptides or

detached envelopes of poxviruses. WO 95/22978, however, does not disclose any particular viral polypeptides other than the viral fusion protein and adsorption protein. Moreover, WO 95/22978 teaches that the disclosed paramunity inducers have virtually no immunogenic properties. There is a need in the art for simple, effective therapeutic agents that can be used to enhance immune responses and to treat infectious diseases and cell proliferative disorders, including tumors and dysplastic lesions.

SUMMARY OF THE INVENTION

[06] It is an object of the invention to provide reagents and methods for modifying immune responses to endogenous (nonadministered) antigens. This and other objects of the invention are provided by one or more of the embodiments described below.

[07] It is an object of the invention to provide a method of enhancing an immune response to endogenous antigens, comprising the steps of administering to a subject in need thereof an effective amount of a B2L viral envelope protein of a *Parapox* virus, whereby the B2L viral envelope protein enhances the immune response to endogenous antigens.

[08] It is an object of the invention to provide a method of eliciting an immune response to B2L protein, comprising the steps of administering to a subject in need thereof an effective amount of a B2L viral envelope protein of a *Parapox* virus, whereby an immune or inflammatory response to the B2L viral envelope protein is generated.

[09] Another embodiment of the invention provides a method of enhancing an immune response to endogenous antigens comprising the steps of administering to a mammal or more specifically a human in need thereof an effective amount of a B2L viral envelope protein of a *Parapox* virus wherein the B2L protein is administered to the subject by means of a nucleotide encoding the B2L protein.

[10] Another embodiment of the invention provides a method of enhancing an immune response to endogenous antigens comprising the steps of administering to a mammal or more specifically a human in need thereof an effective amount of a B2L viral envelope protein of a *Parapox* virus, wherein the *Parapox* virus is *Parapoxvirus ovis*, or more specifically a *Parapoxvirus ovis* strain selected from the group consisting of NZ2, NZ7, NZ10, and D1701.

[11] Another embodiment of the invention provides a method of enhancing an immune response to endogenous antigens, comprising the steps of administering to a subject in need thereof an effective amount of a B2L viral envelope protein of a *Parapox* virus, whereby the B2L viral envelope protein enhances the immune response to endogenous antigens, wherein the endogenous antigens are associated with a cell proliferative disorder or an infectious pathogen.

[12] Another embodiment of the invention provides a method of enhancing an immune response to endogenous antigens, comprises the steps of administering intradermally, subcutaneously, intratumorally, or intravenously to a subject in need thereof an effective amount of a B2L viral envelope protein of a *Parapox* virus.

[13] In yet another embodiment of the invention, a method is provided for treating a patient having a cell proliferative disorder, comprising the step of administering to the patient an effective amount of a B2L viral envelope protein of a *Parapox* virus, whereby symptoms of the patient's cell proliferative disorder are ameliorated.

[14] In yet another embodiment of the invention, a method is provided for treating a patient having a cell proliferative disorder, comprising the step of administering systemically or by injection into a tumor or dysplastic lesion of a patient an effective amount of a B2L viral envelope protein of a *Parapox* virus, whereby symptoms of the patient's cell proliferative disorder are ameliorated.

[15] In yet another embodiment of the invention, a pharmaceutical composition is provided that comprises a B2L viral envelope protein of a *Parapox* virus.

[16] In yet another embodiment of the invention, a pharmaceutical composition is provided that comprises a B2L viral envelope protein a *Parapoxvirus ovis* strain selected from the group consisting of NZ2, NZ7, NZ10, and D1701.

[17] In yet another embodiment of the invention, a method is provided for identifying test compounds with the ability to modify a subject's immune and/or inflammatory response to B2L protein, comprising the steps of: contacting a dendritic cell *in vitro* with a sufficient amount of a purified B2L protein to observe a chemotactic effect of the B2L protein on the dendritic cell; and contacting the dendritic cell with the test compound, whereby a test compound that enhances the chemotactic effect of the B2L protein on the dendritic cell is identified as a potential agent for enhancing the subject's immune response to the B2L protein and whereby a test compound that decreases the chemotactic effect of the B2L protein on the dendritic cell is identified as a potential agent for inhibiting the subject's immune response to the B2L protein.

[18] In yet another embodiment of the invention, a method is provided for identifying test compounds with the ability to modify a subject's immune and/or inflammatory response to B2L protein, comprising the steps of simultaneously contacting a dendritic cell *in vitro* with a sufficient amount of a purified B2L protein and a test compound to observe a chemotactic effect of the B2L protein and test compound on the dendritic cell to identify as a potential agent for enhancing the subject's immune response to the B2L protein.

[19] In still another embodiment of the invention, a method is provided for identifying a test compound with the ability to modify a subject's immune response to a B2L protein, comprising the steps of contacting a purified B2L protein *in vitro* with a test compound; and determining whether the test compound and the B2L protein form a complex,

whereby a test compound that forms a complex with the B2L protein is identified as a potential agent for modifying the subject's immune response to the B2L protein.

[20] In yet another embodiment of the invention, a method is provided for identifying test compounds with the ability to modify a subject's immune and/or inflammatory response to B2L protein, comprising the steps of contacting a peripheral blood mononuclear cell *in vitro* with a sufficient amount of a purified B2L protein to observe a cytokine-inducing effect of the B2L protein on the peripheral blood mononuclear cells, whereby a test compound that enhances the cytokine-inducing effect of the B2L protein on the mononuclear cell is identified as a potential agent for enhancing the subject's immune response to the B2L protein and whereby a test compound that decreases the cytokine-inducing effect of the B2L protein on the mononuclear cell is identified as a potential agent for inhibiting the subject's immune response to the B2L protein.

[21] Thus, the invention provides pharmaceutical compositions and methods for using B2L protein as a monotherapeutic agent, as well as methods of using B2L protein to identify agents that can modify the effects of B2L protein.

BRIEF DESCRIPTION OF THE FIGURES

[22] FIG. 1. Chemotactic effect of B2L protein on human dendritic cell-enriched cultures of peripheral blood mononuclear cells.

[23] FIG. 2. Chemotactic effect of B2L on murine dendritic cell-enriched cultures of bone marrow cells.

[24] FIG. 3. Local and systemic anti-tumor effect of intratumorally administered B2L protein.

[25] FIG. 4. Immune dependence of local and systemic anti-tumor effect of intratumorally administered B2L protein.

[26] FIG. 5. B2L augments IFNγ production by human PBMC

[27] FIG. 6 Subcutaneous and intraperitoneal administration of tagged B2L results in tumor growth inhibition

[28] FIG. 7 Subcutaneous administration of untagged B2L results in tumor growth inhibition

[29] FIG. 8. Intravenous administration of untagged B2L results in tumor growth inhibition

[30] FIG. 9. Systemic administration of B2L results in tumor growth inhibition in multiple tumor models and mouse strains

DETAILED DESCRIPTION OF THE INVENTION

[31] The invention is based on the ability of a *Parapox* viral envelope protein termed "B2L" to enhance a subject's immune response to endogenous (nonadministered) antigens. B2L is the second open reading frame in the *Bam*HI B fragment of the Orf virus genome. Sullivan *et al.*, *Virology 202*, 968-73, 1994. B2L is an immunogenic protein; in fact, B2L protein is the dominant focus of the antibody response mounted in infected sheep. Sullivan *et al.*, 1994. The prior art teaches that, as the activity of epitopes responsible for antigen-specific immunization decrease, nonspecific immunostimulatory activity of the preparations increases. See WO 95/22978, page 4. Thus, immunogenicity of B2L does not predict its ability to modulate immune responses to endogenous antigens. While not wishing to be bound by any particular hypothesis, we believe that B2L, like chemokines, recruits dendritic cells to the site of administration and induces immunity. The ability of B2L to recruit dendritic cells to the injection site promotes activation of helper T cells and cytotoxic T lymphocytes specific for endogenous antigens expressed at the site. In support of this hypothesis, purified B2L protein from mammalian cells transfected with B2L is chemotactic *in vitro* for human dendritic cell-enriched populations (Example 1 and FIG. 1). Purified B2L protein also exhibits chemotactic activity on murine dendritic

cell-enriched cultures with a potency greater than or equal to that observed with secondary lymphoid chemokine (SLC), a chemokine reported to recruit dendritic cells *in vitro* and *in vivo* (Example 2 and FIG. 2). Upon intratumoral injection, purified B2L protein inhibits tumor growth (Example 3 and FIG 3) in an immune dependent fashion (Example 4 and FIG 4).

[32] As has been suggested for other dendritic cell chemotaxins, the chemotactic function of B2L suggests that organization of a local immunologic microenvironment plays a role in its immunostimulatory mechanism of action. Conversely, systemic administration would not be expected to lead to such local organization and, if that were the sole activity of B2L, would not be expected to exhibit antitumor activity. The parent virus has been demonstrated to elicit nonspecific immunostimulatory effects, believed to be cytokine mediated, when administered systemically. Since the virus expresses multiple immunologically relevant proteins, it was not clear which proteins might account for the systemic effect. To our knowledge, activities beyond dendritic cell recruitment and its immunologic consequences have not been demonstrated for B2L prior to the studies described herein. These studies demonstrate that B2L augments production by human PBMC of IFNγ (Example 5 and FIG. 5), a cytokine with known antitumor activity and exhibits robust antitumor activity when administered systemically (Examples 6, 7, 8 and FIGs 6, 7, 8). While not wishing to be bound by any particular hypothesis, we believe that B2L, like its parent virus, nonspecifically stimulates production of IFNγ when administered systemically. IFNγ may both promote development of an antitumor immune response by facilitating the generation of cytotoxic effector cells, by increasing the expression of major histocompatability complex (MHC) antigens on tumors, rendering them more susceptible to being destroyed by cytotoxic effector cells, and by facilitating the generation of tumor-specific antibodies.

[33] Treatment with the highly attenuated parent virus promotes immunity to heterologous pathogens, an effect believed to depend on the induction of multiple cytokines. While not

wishing to be bound by any particular hypothesis, we believe that B2L, like its parent virus, nonspecifically stimulates production of multiple cytokines when administered systemically. Therefore, treatment with the B2L protein may similarly promote immunity to heterologous pathogen.

B2L Proteins

[34] B2L proteins for use in the compositions and methods described herein are those of the *Parapoxvirus* genus, such as Orf virus (OV), particularly the *Parapoxvirus ovis* strains NZ2, NZ7, NZ10, and D1701. Orf viruses are reviewed in Robinson & Balassu, *Vet. Bull. 51*, 771, 1981; Robinson & Lyttle, in Binns & Smith, eds., RECOMBINANT POXVIRUSES, Chapter 9, pp. 306-17, CRC Press, Boca Raton, 1992. An amino acid sequence for the B2L protein of OV NZ2 is disclosed in Sullivan *et al.*, Identification and characterization of an orf virus homologue of the vaccinia virus gene encoding the major envelope antigen p37K, *Virology 202* (2), 968-73, 1994, and is shown in SEQ ID NO:1. A coding sequence for SEQ ID NO:1 is shown in SEQ ID NO:2. The amino acid sequences of the B2L proteins obtained from D1701 and NZ2 are highly conserved. The amino acid sequence of the D1701 protein is shown in SEQ ID NO: 3. A coding sequence for SEQ ID NO: 3 is shown in SEQ ID NO: 4.

[35] Purified B2L protein is separated from other compounds that normally associate with the B2L protein in the virus, such as other envelope components. A preparation of purified B2L protein is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis.

[36] Purified B2L protein for use in compositions and methods of the invention can be purified from *Parapox* viruses or from cells infected by the viruses, by recombinant DNA methods, and by chemical synthesis. Purification methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange

chromatography, affinity chromatography, and preparative gel electrophoresis.

[37] B2L protein can be expressed recombinantly, after insertion of B2L coding sequences into an expression vector that contains the necessary elements for the transcription and translation of the inserted coding sequence. Maintenance of orf viruses in culture is disclosed in WO 97/37031. A preferred system for maintaining and expressing B2L protein is HKB11 cells transfected with B2L in a vector such as a p2ToP, pCEP4, or pcDNA3.1 vector (Invitrogen). Recombinantly produced B2L protein can be secreted into the culture medium and purified. Methods for producing proteins recombinantly are well known to those skilled in the art.

[38] A B2L protein also can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc. 85*, 2149-2154, 1963; Roberge *et al.*, *Science 269*, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation. Optionally, fragments of a B2L protein can be separately synthesized and combined using chemical methods to produce a full-length molecule.

[39] "B2L protein" as used herein includes both functional portions of B2L and full-length or partial biologically active B2L variants. Biologically active variants comprise amino acid substitutions, insertions, and/or deletions with respect to the amino acid sequence shown in SEQ ID NO:1. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine. Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of a B2L protein can be found using computer programs well known in the art, such as DNASTAR software.

Biological activity of a B2L protein having an amino acid substitution, insertion, and/or deletion can be tested, for example, using the *in vitro* assays described in Examples 1 and 2.

[40] Functional portions of B2L comprising, for example, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 360, 370, 375, or 377 amino acids, also can be used in the compositions and methods of the invention, provided that the portions of B2L retain biological activity, *e.g.*, the ability to enhance an immune response and/or exert a chemotactic effect on enriched dendritic cell populations and/or exert a cytokine-inducing effect on peripheral blood mononuclear cells.

Pharmaceutical compositions

[41] Purified B2L protein can be used in pharmaceutical compositions. Pharmaceutical compositions of the invention can be used to produce tumor and pathogen protection in mammals, including laboratory animals (*e.g.*, mice, rats, hamsters, guinea pigs), companion animals (*e.g.*, dogs, cats), farm animals (*e.g.*, horses, cows, sheep, pigs, goats), and humans.

[42] Pharmaceutical compositions of the invention include a pharmaceutically acceptable carrier. Typically these will be sterile formulations in a diluent or vehicle which is free of pyrogenic components. Buffers, stabilizers, and the like can be included, as is known in the art. Optionally, pharmaceutical compositions include conventional adjuvants, such as aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum), saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria in mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide, and lipopolysaccharides.

[43] B2L-containing compositions can be administered alone or can be co-administered with one or more other therapeutic agents, such as a tumor-specific antibody or cytokine. "Co-administration" includes administration of B2L and another therapeutic agent separately or in the same composition.

[44] A large number of antibodies have been described which are specifically reactive with tumor-associated antigens. Many are available from the American Type Culture Collection, 10801 University Blvd., Manassas, Virginia 20110-2209, including those to breast, lung, and melanoma tumor cells. For a review of tumor-specific antibodies see Foon, *Cancer Research 49*, 1621-31, 1989.

[45] Endogenous, nonadministered antigens comprise tumor antigens and/or those produced by natural infection with a pathogen. Antigens include any component that is recognized by cells of the immune system. Endogenous tumor antigens include, but are not limited to, α-fetoprotein, BAGE, β-HCG, CEA, ESO, GAGE, gangliosides, Her-2/neu, HPV E6/E7, immunoglobulins, MAGE-1, MAGE-2, MAGE-3, MAGE-4, MAGE-12, MART-1, Melan-A, melanoma antigen gp75, gp100, MN/G250, MUC1, MUC2, MUC3, MUC4, MUC18, PSA, PSM, RAGE, ras, SART-1, telomerase, thyroperoxidases, tyrosinases, and p53. Endogenous pathogen-associated antigens include, but are not limited to, those produced by natural infection with human immunodeficiency viruses, *Herpes* viruses, hepatitis viruses, pox viruses, flu viruses, measles, mumps, rubella, rabies, respiratory syncytial viruses, *Bacillus anthracis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Clostridium tetani*, *Corynebacterium diphtheriae*, *Haemophilus influenza B*, *Neisseria meningitidis*, *Salmonella typhi*, *Streptococcus pneumoniae*, and *Vibrio cholerae*.

[46] Routes of administration include, without limitation, subcutaneous, intravenous, nasal, ophthalmic, transdermal, intramuscular, intradermal, intragastric, perlingual, alveolar, gingival, intraperitoneal, intravaginal, pulmonary, rectal, and oral administration.

Administration can be by any suitable means, including injection, topical administration, ingestion, or inhalation. Single and/or multiple administrations are contemplated.

[47] Optionally, B2L protein can be administered using a nucleotide (DNA or RNA) molecule encoding the protein. Use of DNA-encoded elicitors of immune responses is discussed, for example, in McDonnel & Askari, *New Engl. J. Med. 334*, 42-45, 1996; Robinson, *Can. Med. Assoc. J. 152*, 1629-32, 1995; Fynan *et al.*, *Int. J. Immunopharmacol. 17*, 79-83, 1995; Pardoll & Beckerleg, *Immunity 3*, 165-69, 1995, and Spooner *et al.*, *Gene Therapy 2*, 173-80, 1995.

Dosage

[48] The determination of a therapeutically effective dose of B2L is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient (*i.e.*, B2L protein or nucleic acid encoding B2L) that results in an augmentation of the immune response to cancer or an endogenous antigen over that which occurs in the absence of the therapeutically effective dose.

[49] The therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rats, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

[50] Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}.

[51] Pharmaceutical compositions that exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

[52] Suitable dosages and treatment regimens for administration of either B2L protein or nucleic acid molecules encoding B2L include, but are not limited to, daily, twice- or three-times weekly, weekly, bi-weekly, monthly, bimonthly, or yearly treatments of about 1, 5, 10, 15, 20, 25, 50, 75, 100, or 200 $\mu g/m^2$ (or approximately 0.05, 0.1, 0.2, 0.25, 0.3, 0.5, 0.75, 1, 2, 5, or 10 mg/kg). Preferred routes of administration include subcutaneous, intradermal, intramuscular administration. The exact dosage, treatment regimen, and route of administration, however, will be determined by the practitioner, in light of factors related to the subject that requires treatment.

Treatment of cell proliferative disorders

[53] In one embodiment, the invention provides a therapeutic agent for cell proliferative disorders. A "cell proliferative disorder" includes both benign and malignant cell populations that differ morphologically from the surrounding tissue. A cell proliferative disorder as described herein may be a neoplasm. A neoplasm is a new, abnormal growth of cells or a growth of abnormal cells that reproduce faster than normal. A neoplasm can create an unstructured mass (a tumor), which can be either benign or malignant. For example, the neoplasm may be a brain, head, neck, skin, lung, esophageal, stomach, small bowel, colon, bladder, kidney, or cervical neoplasm. A benign tumor is a tumor that is noncancerous, *e.g.*, its cells do not proliferate or invade surrounding tissues. A malignant tumor comprises cells that may become metastatic. A neoplasm also includes

an abnormal proliferation of blood cells such as occurs in leukemias, lymphomas, polycythemia vera, thrombocytosis, and the like.

[54] Cell proliferative disorders also include disorders such as anhydric hereditary ectodermal dysplasia, congenital alveolar dysplasia, epithelial dysplasia of the cervix, fibrous dysplasia of bone, and mammary dysplasia. Cell proliferative disorders also include hyperplasias, for example, endometrial, adrenal, breast, prostate, thyroid hyperplasias, and pseudoepitheliomatous hyperplasia of the skin.

[55] For treatment of neoplasms or other cell proliferative lesions, pharmaceutical compositions of the invention preferably are administered by injection. For example, B2L protein itself can be injected directly into the abnormal tissue or into the surrounding tissue, although other means of administration can be used. If desired, conjugates of B2L protein with, *e.g.*, a tumor-specific antibody, preferably are administered systemically.

Screening methods

[56] The invention also provides methods of screening test compounds to identify those which are potential agents for modulating an immune response, particularly an individual's immune response to B2L.

[57] Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead

one-compound" library method, and synthetic library methods using affinity chromatography selection.

[58] Methods for the synthesis of molecular libraries are well known in the art (*see*, for example, DeWitt *et al., Proc. Natl. Acad. Sci. U.S.A. 90*, 6909, 1993; Erb *et al. Proc. Natl. Acad. Sci. U.S.A. 91*, 11422, 1994; Zuckermann *et al., J. Med. Chem. 37*, 2678, 1994; Cho *et al., Science 261*, 1303, 1993; Carell *et al., Angew. Chem. Int. Ed. Engl. 33*, 2059, 1994; Carell *et al., Angew. Chem. Int. Ed. Engl. 33*, 2061; Gallop *et al., J. Med. Chem. 37*, 1233, 1994). Libraries of compounds can be presented in solution (*see, e.g.*, Houghten, *BioTechniques 13*, 412-421, 1992), or on beads (Lam, *Nature 354*, 82-84, 1991), chips (Fodor, *Nature 364*, 555-556, 1993), bacteria or spores (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al., Proc. Natl. Acad. Sci. U.S.A. 89*, 1865-1869, 1992), or phage (Scott & Smith, *Science 249*, 386-390, 1990; Devlin, *Science 249*, 404-406, 1990); Cwirla *et al., Proc. Natl. Acad. Sci. 97*, 6378-6382, 1990; Felici, *J. Mol. Biol. 222*, 301-310, 1991; and Ladner, U.S. Patent 5,223,409).

[59] Screening assays of the invention can employ test compounds together with in chemotaxis assays, such as those disclosed in Examples 1 and 2 or in cytokine induction assays, such as that disclosed in Example 5. Alternatively, purified B2L protein can be contacted with test compounds *in vitro* and the formation of a complex between B2L and the test compound determined. Optionally, either the B2L protein or the test compound can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, to facilitate detection of B2L-test compound complexes.

[60] Any means known in the art can be used to determine whether a B2L-test compound complex has been formed. Such methods include, without limitation, yeast two-hybrid assays (*e.g.*, U.S. Patent 5,283,317; Zervos *et al., Cell 72*, 223-232, 1993; Madura *et al., J. Biol. Chem. 268*, 12046-12054, 1993; Bartel *et al., BioTechniques 14*, 920-924, 1993; Iwabuchi *et al., Oncogene 8*, 1693-1696, 1993; and Brent W094/10300), real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem. 63*,

2338-2345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol.* *5*, 699-705, 1995), and detection of complexes via non-denaturing SDS polyacrylamide gel electrophoresis.

[61] B2L induces distinct immunologic effects. Because it is a foreign protein, it is recognized as an antigen by T and B lymphocytes with antigen receptors specific for B2L. It also activates antigen nonspecific cells including, but not limited to dendritic cells which do not express antigen specific receptors and thereby causing antigen-nonspecific inflammatory responses. It may be desirable to elicit a nonspecific inflammatory response to the B2L protein without a concomitant specific immune response to the protein. Development of an inflammatory response can be determined by evaluating serum or plasma from treated individuals for the presence of proinflammatory cytokines including, but not limited to TNFα, IL-1β, IL-6, and IFNγ. Additionally, development of an inflammatory response can be determined by evaluating peripheral blood mononuclear cells or the tissue at the injection site for increased numbers of monocytes, granulocytes, lymphocytes or neutrophils. Development of a specific humoral immune response to B2L can be determined by evaluating serum or plasma from treated individuals for the presence of antibodies that specifically recognize B2L. Additionally, development of a cell mediated immune response to B2L can be determined by evaluating the selective proliferation of and/or cytokine production by peripheral blood mononuclear cells or isolated lymphocytes in response to *in vitro* exposure to the B2L protein

[62] It may be desirable to immobilize either the B2L protein or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the B2L or the test compound can be bound to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the B2L protein or the test

compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the B2L protein or test compound and the solid support. Test compounds are preferably bound to the solid support in an array, so that the location of individual test compounds can be tracked. Binding of a test compound to a B2L can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

[63] All patents and patent applications cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples, which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Chemotaxis of human dendritic cells in response to B2L

[64] Proteins that are chemotactic for dendritic cells would be useful as single agent therapeutics in cancer patients (Fushimi et al. J. Clin. Invest. 2000. 105:1383; Sharma et al. J Immunol. 2000. 164:4558). *Parapoxvirus ovis* causes dendritic cell recruitment. To determine if the isolated B2L protein of the parent virus exhibited similar chemotactic activity, human peripheral blood mononuclear cells were cultured with GM-CSF and IL-4 to allow differentiation of dendritic cells. After labeling with Calcein-AM dye, 10^5 cells were loaded into the upper compartment of chemotaxis culture chambers and the indicated concentrations of RANTES chemokine or purified V5His6-tagged, Strep-tagged or untagged B2L protein loaded into the lower compartment. Cell migration through a cell-permeable membrane separating the two compartments was assessed after 2hr 15min. The results shown in FIG. 1 confirm published reports that RANTES chemokine causes migration of human dendritic cells. Similarly, dendritic cells migrate in response to V5His6-tagged B2L, albeit to a lesser extent than to RANTES in the experiment shown. Heat inactivation of the protein resulted in loss of chemotactic activity, demonstrating that activity was not a function of trace levels of endotoxin in the preparation (not shown). The chemotactic activity is a function of the B2L protein itself rather than of the V5His6 epitope tag, as demonstrated by comparable activity of the untagged molecule. These results suggest that B2L administered intratumorally, as a single agent would recruit dendritic cells to the site of tumor antigen. This would facilitate uptake of endogenous tumor antigens by the dendritic cells and subsequent induction of a systemic tumor specific immune response.

EXAMPLE 2

Chemotaxis of murine dendritic cells in response to B2L

[65] Proof of concept studies were designed in murine tumor models. Murine secondary lymphoid chemokine (mSLC) is known to elicit an antitumor immune response when administered intratumorally. The doses needed for this activity have been published. To select a range of doses of V5His6-tagged B2L likely to exhibit antitumor activity in mice, we compared the amount of tagged B2L needed for migration of murine dendritic cells with the amount of mSLC needed. Murine bone marrow cells were cultured with GM-CSF and IL-4 for 5 days to enrich for dendritic cells. After labeling with Calcein-AM dye, 10^5 cells were loaded into the upper compartment of chemotaxis culture chambers and the indicated concentrations of murine SLC chemokine or purified V5His6-tagged B2L protein into the lower compartment. Cell migration through a cell-permeable membrane separating the two compartments was assessed after 2hr 15min. The results shown in FIG. 2 depict a relatively shallow dose response curve for both proteins but indicate that the chemotactic activity of the tagged B2L is at least as potent as that of mSLC and suggested that equivalent doses of both proteins intratumorally might exhibit antitumor activity.

EXAMPLE 3

Local and systemic antitumor activity of intratumorally administered B2L

[66] Local antitumor activity had previously been reported for intratumorally administered dendritic cell chemoattractants. Therapeutic application would be severely limited if activity were confined to the accessible tumor. Therefore, therapeutic efficacy of tagged B2L was evaluated in a double-implant model to allow evaluation not only of local efficacy but systemic efficacy as well. B16.F10 murine melanoma cells were implanted both subcutaneously and intravenously, the former providing a tumor accessible to drug administration and the latter leading to development of pulmonary tumor nodules distant to the site of drug administration. Intratumoral administration of V5His6-tagged B2L into the subcutaneous tumor nodule, indicated by the black arrows, began on day 6 when nodules became palpable. As expected, based on published literature, tumor growth measured on day 21 was inhibited by mSLC at both subcutaneous (FIG 3A) and pulmonary sites (FIG 3B) by 50% ($p<0.001$) and 39% ($p<0.02$), respectively. Similarly, intratumorally administered tagged B2L inhibited growth of the injected subcutaneous tumor by 55% ($p<0.01$) and the development of pulmonary nodules by 53% ($p<0.01$). These results indicate that tagged B2L exhibits antitumor activity comparable to that of mSLC. Furthermore, administration of tagged B2L resulted in growth inhibition even when administered at a site distant to the tumor. This result is consistent with the possibility that intratumoral administration of tagged B2L led to development of a systemic antitumor immune response.

EXAMPLE 4

Immune dependence of local and systemic antitumor activity of intratumorally administered B2L

[67] To evaluate the potential role of the cell-mediated immunity in the antitumor activity found for tagged B2L, efficacy was compared in normal immunocompetent mice and in mice rendered partially immunodeficient by depletion of T and natural killer (NK)

lymphocytes. B16.F10 tumor was implanted both subcutaneously and intravenously. Administration of V5His6-tagged B2L into the subcutaneous tumor nodule, indicated by the black arrows, began on day 6 when nodules became palpable. Immune depletion of T and NK cells was achieved through IP injections of antibodies, indicated by red arrows, to these cell types. Tumor growth measured on day 21 was inhibited at both subcutaneous (FIG 4A) and pulmonary sites (FIG 4B) by 68% and 48%% ($p<0.001$), respectively, in immunocompetent mice whereas antitumor activity was not observed in T and NK-depleted littermates. These results indicate that the antitumor activity of intratumorally administered tagged B2L is immune dependent.

EXAMPLE 5

B2L augments IFNγ production by human PBMC

[68] *Parapoxvirus ovis* is known to exhibit nonspecific immunostimulatory effects in animals when administered systemically. The mechanism underlying this effect is believed to be cytokine mediated. *Parapoxvirus ovis* has been shown to induce cytokine production from human PBMC. If B2L exhibited similar activity *in vitro*, one could hypothesize that it would exhibit antitumor activity *in vivo* when administered systemically. To our knowledge, this has not been tested. To do so, human PBMC collected from 3 donors were cultured with 1 or 100 ng/ml untagged B2L alone (not shown), 100 ng/ml LPS alone or in combination with the indicated concentrations of B2L. Supernatants were collected after 48 hours and assayed by a bead-based ELISA (Luminex) for the presence of IFNγ, a cytokine known to have antitumor activity. B2L alone exhibited no effect on PBMC within the timeframe of the experiment (not shown). LPS alone elicited only modest production of IFNγ. The addition of B2L led to substantial increases in the level of IFNγ produced by from two of the three donors in the experiment shown. In repeated experiments, the increase in IFNγ production ranged between 2 and 7 fold. The activity was a function of the B2L protein itself and not a tag, since untagged B2L was used in these experiments. The ability of B2L to elicit *in vitro* production of IFNγ raises the possibility that systemic administration of B2L as a single agent might lead to systemic cytokine production and antitumor activity.

EXAMPLE 6

Subcutaneous and intraperitoneal administration of tagged B2L results in tumor growth inhibition

[69] Due to its additional ability to elicit production of cytokines such as IFNγ which are known to have antitumor activity, one can hypothesize that B2L would exhibit antitumor activity if administered systemically as well. To test this hypothesis, V5His-tagged B2L was administered intraperitoneally (IP) (the 2nd, 3rd, and 4th bars, numbered from the left - under the heading "IP") or subcutaneously (SC) (5th, 6th, and 7th bars, numbered from the left - under the heading "SC") once daily for 5 consecutive days beginning the 6th day after intravenous implantation of B16.F10 tumor (see: FIG 6). The results shown in FIG. 6 demonstrate that a daily dose of 0.1 μg given by either route of administration was ineffective. A daily dose of at least 1 μg, however, led to 70 – 80% reduction in the number of pulmonary nodules. This was seen with both routes of administration. Daily doses higher than 1 μg offered no greater therapeutic benefit. Combined with the results in Figure 5, these results support the therapeutic hypothesis that systemic administration of B2L as a single agent would result in tumor growth inhibition in cancer patients.

EXAMPLE 7

Subcutaneous administration of untagged B2L results in tumor growth inhibition

[70] To evaluate the potential contribution of the epitope tag to the antitumor efficacy found, V5His6-tagged (bar at far right side of histogram, below the number 67) or untagged (the six bars labeled "μg untagged B2L") B2L were administered SC once daily for 5 consecutive days beginning the 6th day after intravenous implantation of B16.F10 tumor (FIG. 7). As observed previously with tagged B2L, the results shown in FIG. 7 demonstrate that untagged B2L is ineffective when administered at a daily dose of 0.1 μg but reduces the number of pulmonary nodules by 50-70% ($p<0.001$) when administered

at daily doses of at least 1 μg. A daily dose of 0.3 μg demonstrated statistically significant efficacy in the experiment shown. This is not a consistently reproducible finding (not shown), indicating a very steep dose response. These results demonstrate that antitumor efficacy is a function of the B2L protein itself, not the epitope tag.

EXAMPLE 8

Intravenous administration of untagged B2L results in tumor growth inhibition

[71] In a limited number of studies, efficacy of untagged B2L was also evaluated after IV administration once daily for 3 consecutive days beginning the 6th day after SC implantation of B16.F10 tumor. The results shown in FIG. 8 demonstrate that a brief 3-day course of therapy with untagged B2L reduces tumor growth by 60-70% ($p<0.05$) when administered at a daily dose of at least 0.3 μg but is ineffective when administered at a dose of 0.1 μg.

EXAMPLE 9

Subcutaneous administration of B2L results in tumor grown inhibition in multiple tumor models and mouse strains

[72] C57BL/6 mice are genetically predisposed to make a Th1 type of immune response, characterized in part by the preferential production of IFNγ. Conversely, BALB/c mice are genetically predisposed to make a Th2 type of immune response, characterized in part by less robust IFNγ responses. The genetic variability of humans also contributes to variability in the types of immune responses they mount. To determine if B2L would exhibit antitumor efficacy in mice of a low IFNγ producing background, untagged B2L was administered SC once daily for 5 consecutive days in BALB/c mice, beginning the 6th day after intravenous implantation of syngeneic CT-26 colon carcinoma cells. The results shown if FIG. 9 (four bars identified as "μg Untreated B2L") demonstrate that a

daily dose of 1 μg, the minimal daily dose that consistently exhibits maximal efficacy in C57BL/6 mice bearing B16.F10 tumors, also exhibits robust antitumor activity in BALB/c mice with established CT-26 tumors. These results suggest that B2L will exhibit antitumor activity in animals and humans of immunologically relevant genetic variability. Due to its effect on dendritic cells and IFNγ production, B2L preparations impact the innate immune system. The innate immune system is highly sensitive to endotoxin, a contaminant frequently found in biologic preparations. The preparations of tagged and untagged B2L used typically contained <1 EU/mg of endotoxin (not shown). Nonetheless, B2L was heat denatured, a process which generally inactivates proteins but does not affect endotoxin. The pale blue bar indicates that heat denatured B2L is inactive, indicating that the activity is a function of the B2L protein itself and not due to trace levels of endotoxin.

We claim:

1. A method of enhancing an immune response to endogenous antigens, comprising the steps of:

   administering to a subject in need thereof an effective amount of a B2L viral envelope protein of a *Parapox* virus, whereby the B2L viral envelope protein enhances the immune response to endogenous antigens.

2. The method of Claim 1 wherein the B2L protein is administered to the subject by means of a nucleic acid coding the B2L protein.

3. The method of Claim 2 wherein the nucleic acid is DNA.

4. The method of Claim 2 wherein the nucleic acid is RNA.

5. The method of Claim 1 wherein the subject is a mammal.

6. The method of Claim 1 wherein the mammal is a human.

7. The method of Claim 1 wherein the *Parapox* virus is *Parapoxvirus ovis*.

8. The method of Claim 1 wherein the *Parapox* virus is a *Parapoxvirus ovis* strain selected from the group consisting of NZ2, NZ7, NZ10, and D1701.

9. The method of Claim 1 wherein the endogenous antigens are associated with a cell proliferative disorder.

10. The method of Claim 1 wherein the endogenous antigens are associated with infectious pathogens.

11. The method of Claim 1 wherein the B2L protein is administered by injection.

12. The method of Claim 1 wherein the B2L protein is administered intradermally, subcutaneously, intranasally, orally, intratumorally, or intravenously.

13. A method of treating a patient having a cell proliferative disorder, comprising the step of:

administering to the patient an effective amount of a B2L viral envelope protein of a *Parapox* virus, whereby symptoms of the patient's cell proliferative disorder are ameliorated.

14. The method of Claim 11 wherein the B2L protein is administered systemically.

15. The method of Claim 11 wherein the B2L protein is injected into a tumor or dysplastic lesion.

16. A method of eliciting in a patient an immune or inflammatory response to B2L protein, comprising the step of:

administering to the patient an effective amount of a B2L viral envelope protein of a *Parapox* virus, whereby an immune or inflammatory response is generated.

17. A pharmaceutical composition comprising:

a B2L viral envelope protein of a *Parapox* virus

18. The pharmaceutical composition of Claim 14 wherein the *Parapox* virus is *Parapoxvirus ovis*.

19. The pharmaceutical composition of Claim 14 wherein the *Parapox* virus is a *Parapoxvirus ovis* strain selected from the group consisting of NZ2, NZ7, NZ10, and D1701.

20. A pharmaceutical composition comprising:
a nucleic acid molecule encoding a B2L viral envelope protein of a *Parapox* virus

21. The pharmaceutical composition of Claim 17 wherein the *Parapox* virus is *Parapoxvirus ovis*.

22. The pharmaceutical composition of Claim 18 wherein the *Parapox* virus is a *Parapoxvirus ovis* strain selected from the group consisting of NZ2, NZ7, NZ10, and D1701.

23. A method of identifying test compounds with the ability to modify a subject's immune and/or inflammatory response to B2L protein, comprising the steps of:

contacting a dendritic cell *in vitro* with a sufficient amount of a purified B2L protein to observe a chemotactic effect of the B2L protein on the dendritic cell; and

contacting the dendritic cell with the test compound, whereby a test compound that enhances the chemotactic effect of the B2L protein on the dendritic cell is identified as a potential agent for enhancing the subject's immune and/or inflammatory response to the B2L protein and whereby a test compound that decreases the chemotactic effect of the B2L protein on the dendritic cell is identified as a potential agent for inhibiting the subject's immune and/or inflammatory response to the B2L protein.

24. The method of Claim 20 wherein the cell is contacted simultaneously with the B2L protein and the test compound.

25. A method of identifying a test compound with the ability to modify a subject's immune and/or inflammatory response to B2L protein, comprising the steps of:

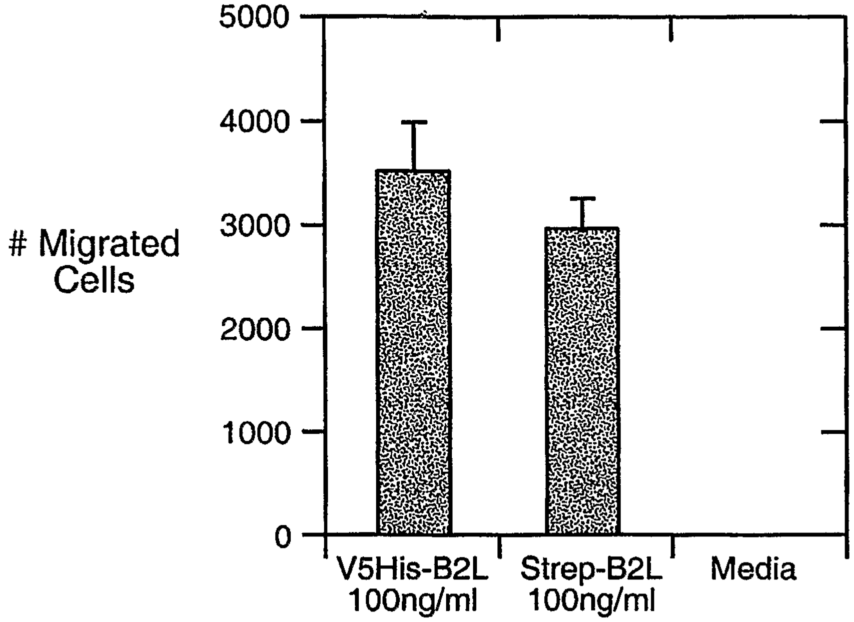
contacting a purified B2L protein *in vitro* with a test compound; and

determining whether the test compound and the B2L protein form a complex, whereby a test compound that forms a complex with the B2L protein is identified as a potential agent for modifying the subject's immune and/or inflammatory response to B2L protein.

26. A method of identifying test compounds with the ability to modify a subject's immune and/or inflammatory response to B2L protein, comprising the steps of:

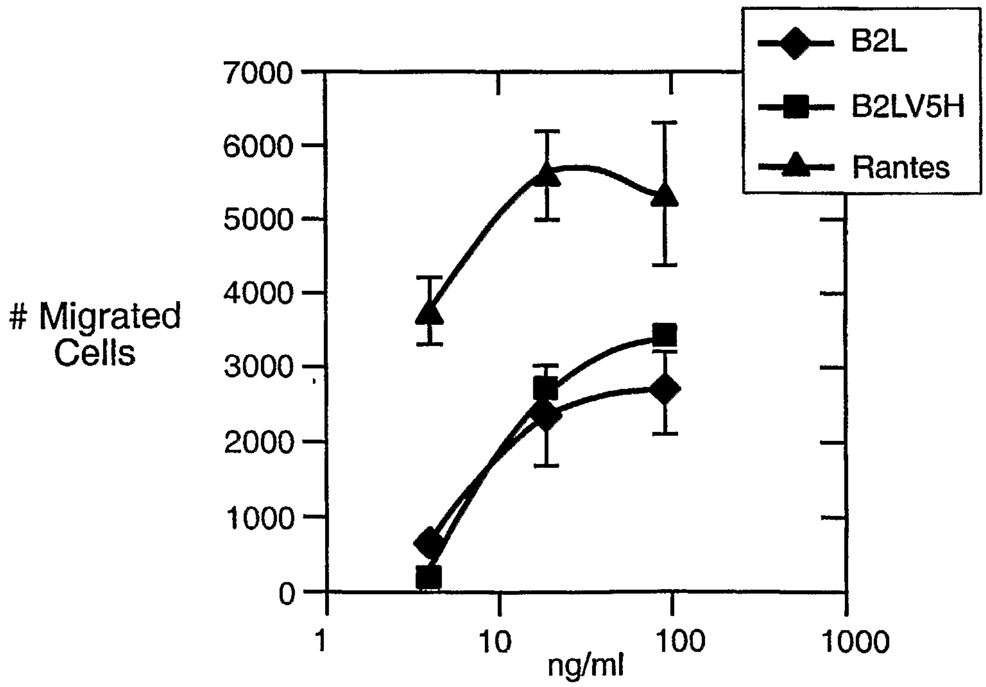
contacting a peripheral blood mononuclear cell *in vitro* with a sufficient amount of a purified B2L protein to observe a cytokine-inducing effect of the B2L protein on the peripheral blood mononuclear cell; and

contacting the peripheral blood mononuclear cell with the test compound, whereby a test compound that enhances the cytokine-inducing effect of the B2L protein on the peripheral blood mononuclear cell is identified as a potential agent for enhancing the subject's immune and/or inflammatory response to B2L protein and whereby a test compound that decreases the cytokine-inducing effect of the B2L protein on the peripheral blood mononuclear cell is identified as a potential agent for inhibiting the subject's immune and/or inflammatory response to B2L protein



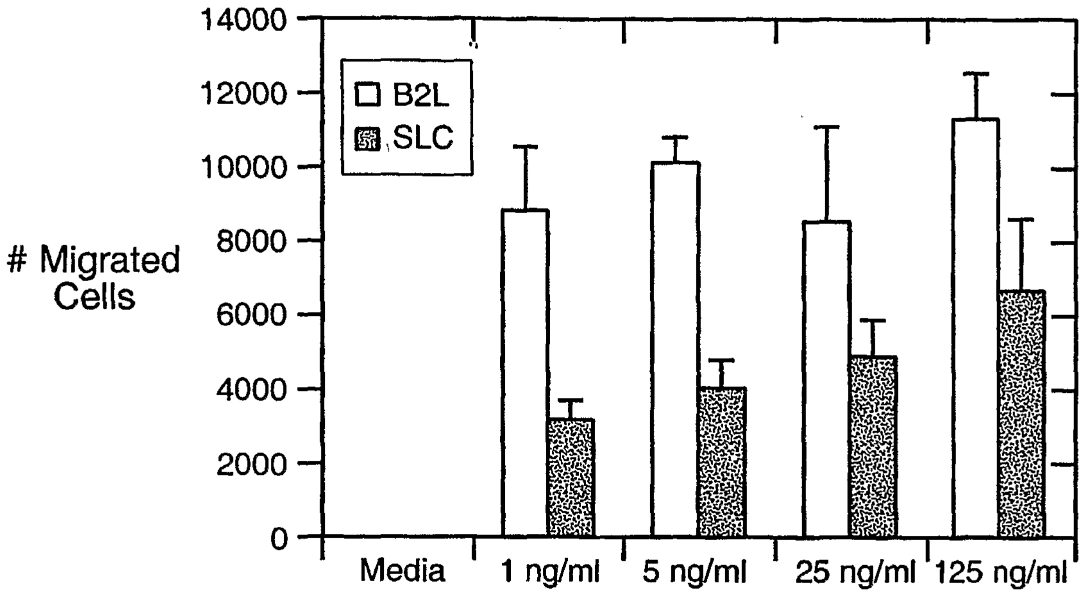
5000
4000
3000
2000
1000
0
Migrated Cells
V5His-B2L 100ng/ml
Strep-B2L 100ng/ml
Media

FIG._1A



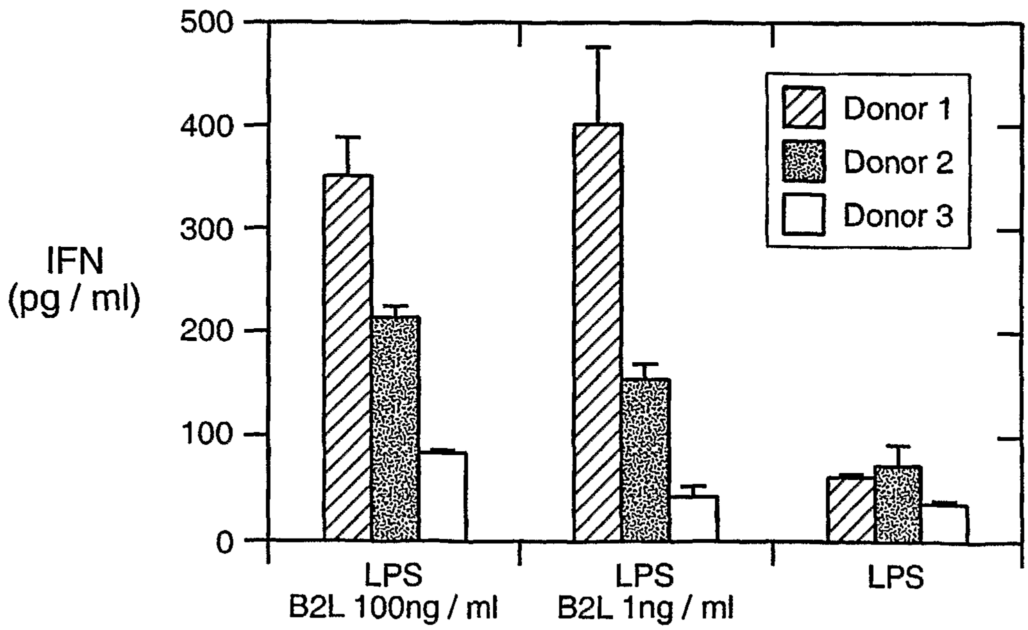
B2L
B2LV5H
Rantes
7000
6000
5000
4000
3000
2000
1000
0
Migrated Cells
1
10
100
1000
ng/ml

FIG._1B



14000
12000
10000
8000
6000
4000
2000
0
Migrated Cells
B2L
SLC
Media
1 ng/ml
5 ng/ml
25 ng/ml
125 ng/ml

FIG._2



500
400
300
200
100
0
IFN (pg / ml)
Donor 1
Donor 2
Donor 3
LPS B2L 100ng / ml
LPS B2L 1ng / ml
LPS

FIG._5

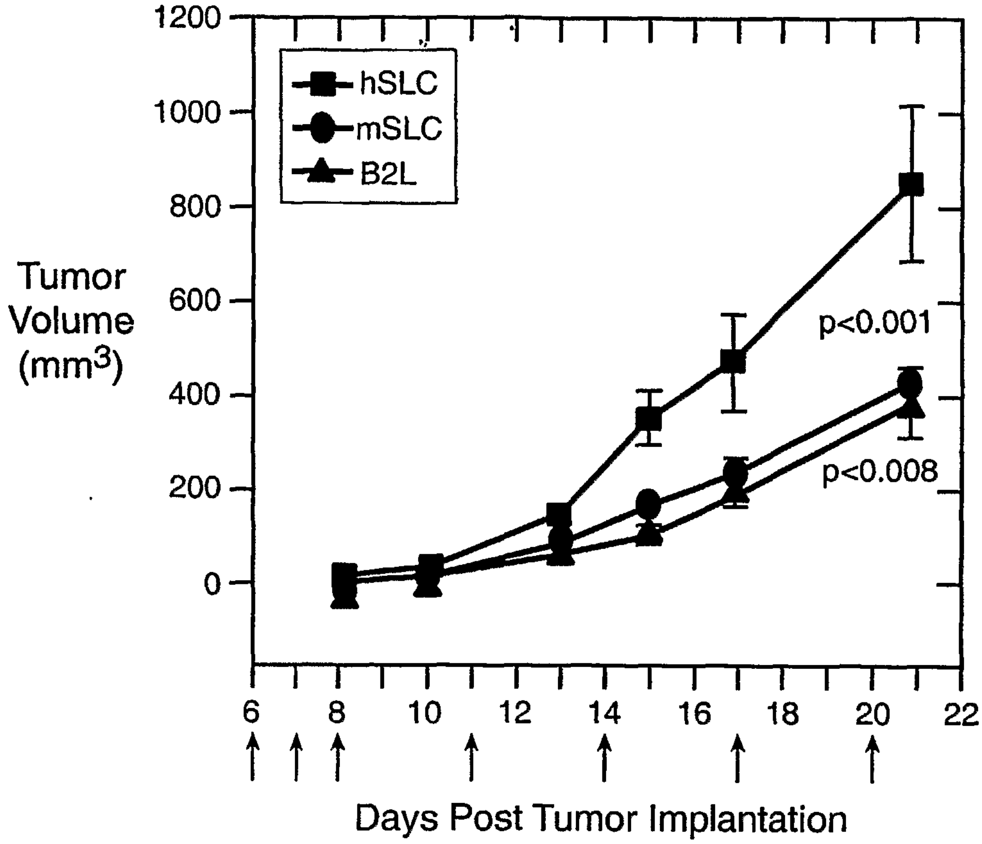

1200
1000
800
600
400
200
0
Tumor Volume (mm3)
hSLC
mSLC
B2L
p<0.001
p<0.008
6
8
10
12
14
16
18
20
22
Days Post Tumor Implantation

FIG._3A

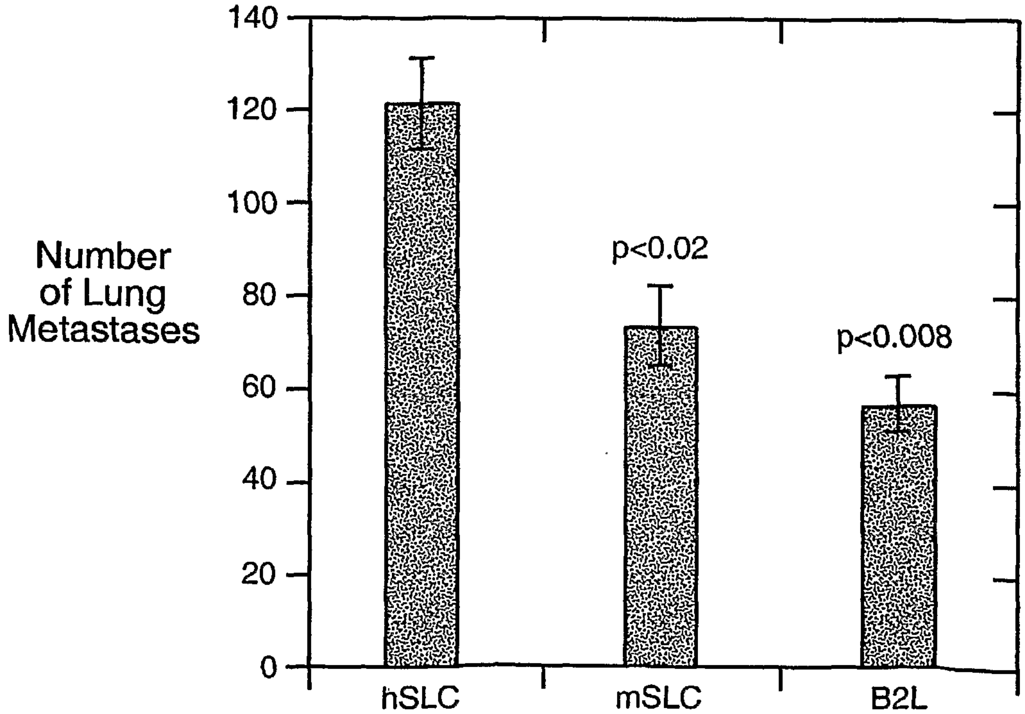

140
120
100
80
60
40
20
0
Number of Lung Metastases
p<0.02
p<0.008
hSLC
mSLC
B2L

FIG._3B

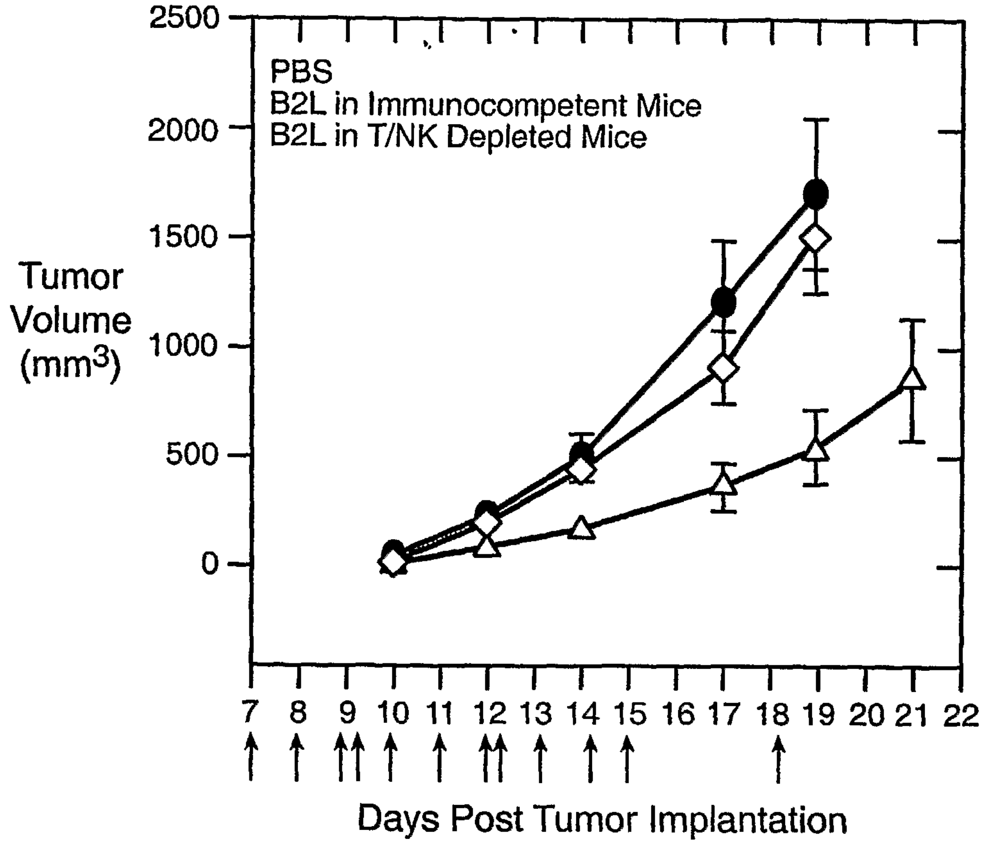
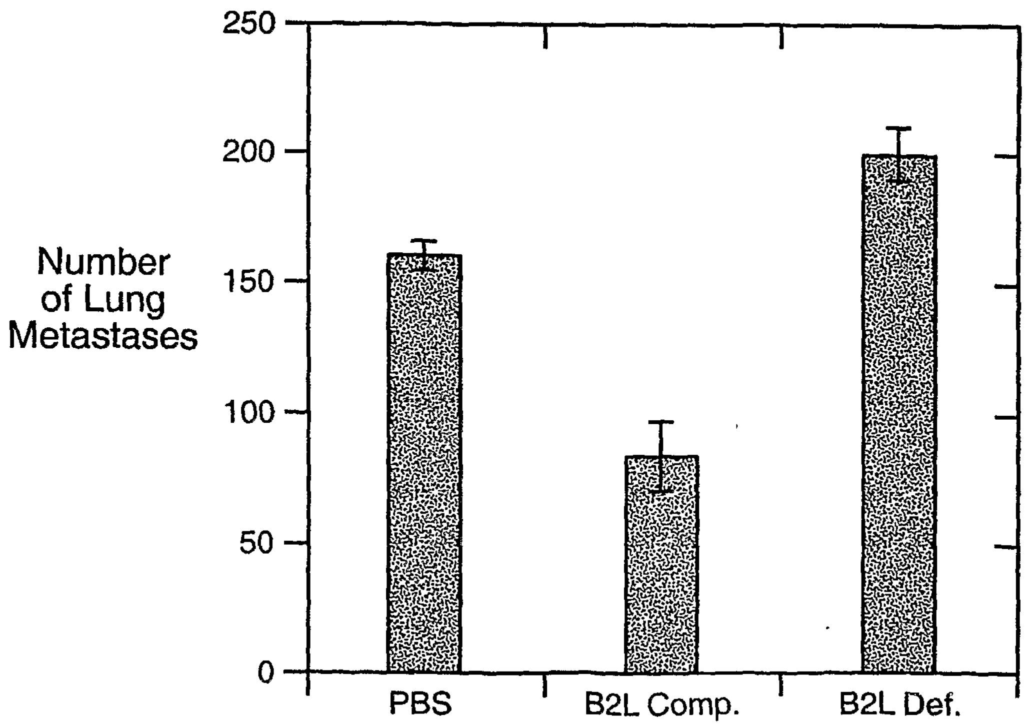

2500
2000
1500
1000
500
0
Tumor Volume (mm3)
PBS
B2L in Immunocompetent Mice
B2L in T/NK Depleted Mice
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days Post Tumor Implantation

FIG._4A


250
200
150
100
50
0
Number of Lung Metastases
PBS
B2L Comp.
B2L Def.

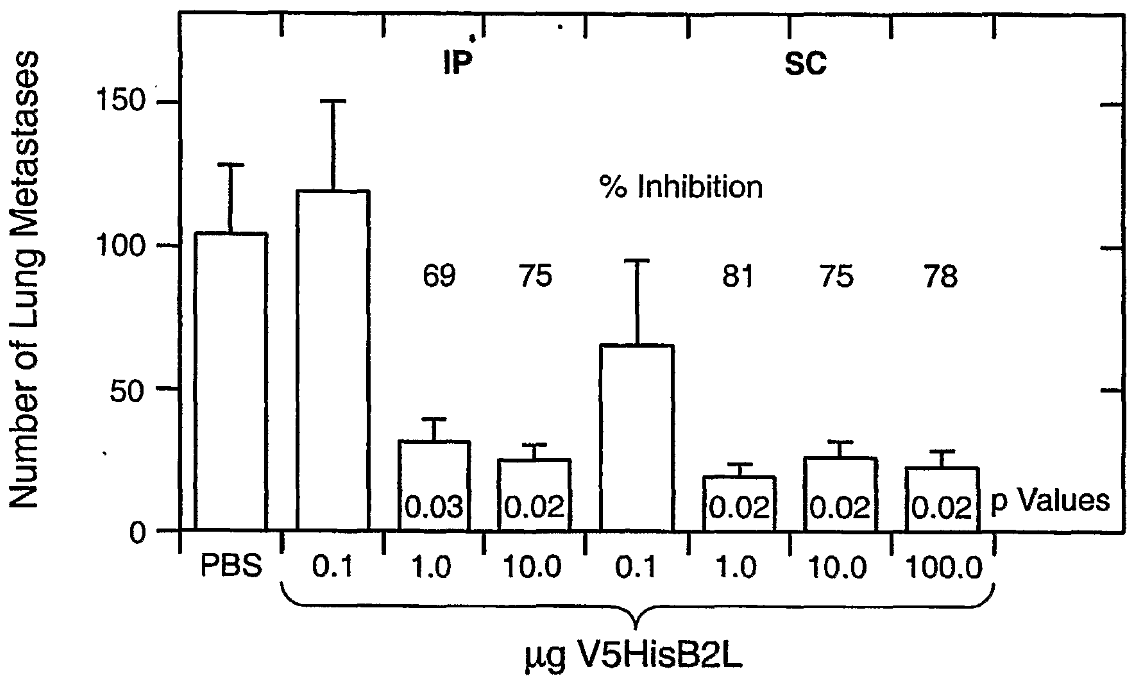

Number of Lung Metastases
150
100
50
0
IP
SC
% Inhibition
69 75 81 75 78
0.03 0.02 0.02 0.02 0.02
p Values
PBS 0.1 1.0 10.0 0.1 1.0 10.0 100.0
μg V5HisB2L

FIG._6

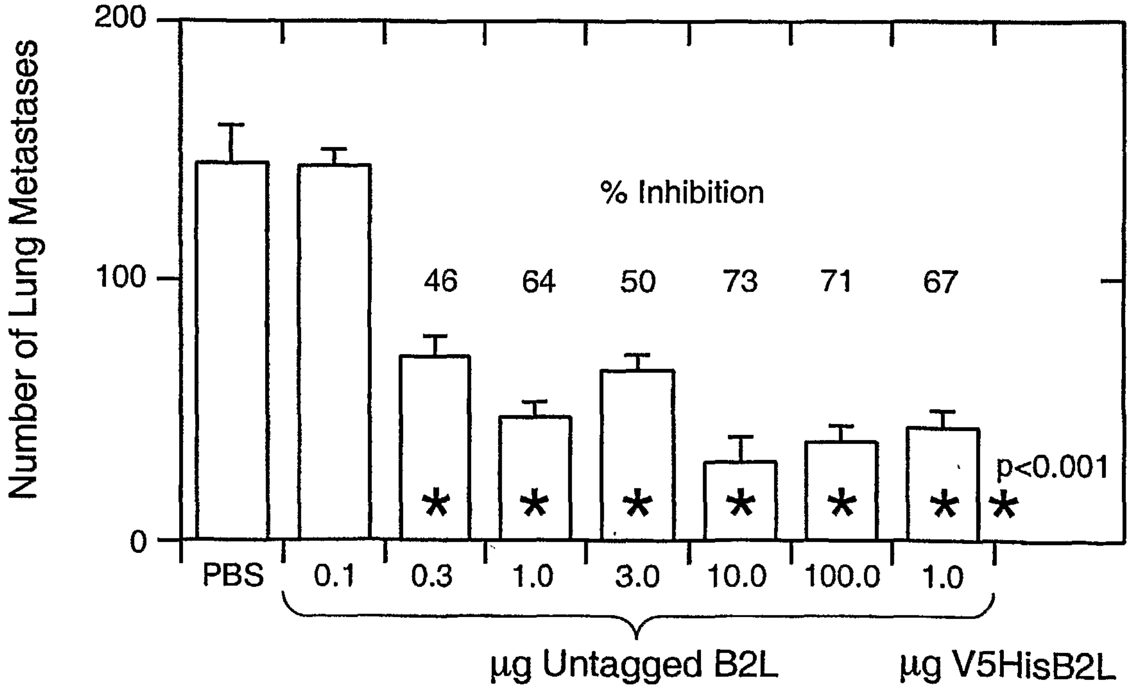

Number of Lung Metastases
200
100
0
% Inhibition
46 64 50 73 71 67
p<0.001
PBS 0.1 0.3 1.0 3.0 10.0 100.0 1.0
μg Untagged B2L
μg V5HisB2L

FIG._7

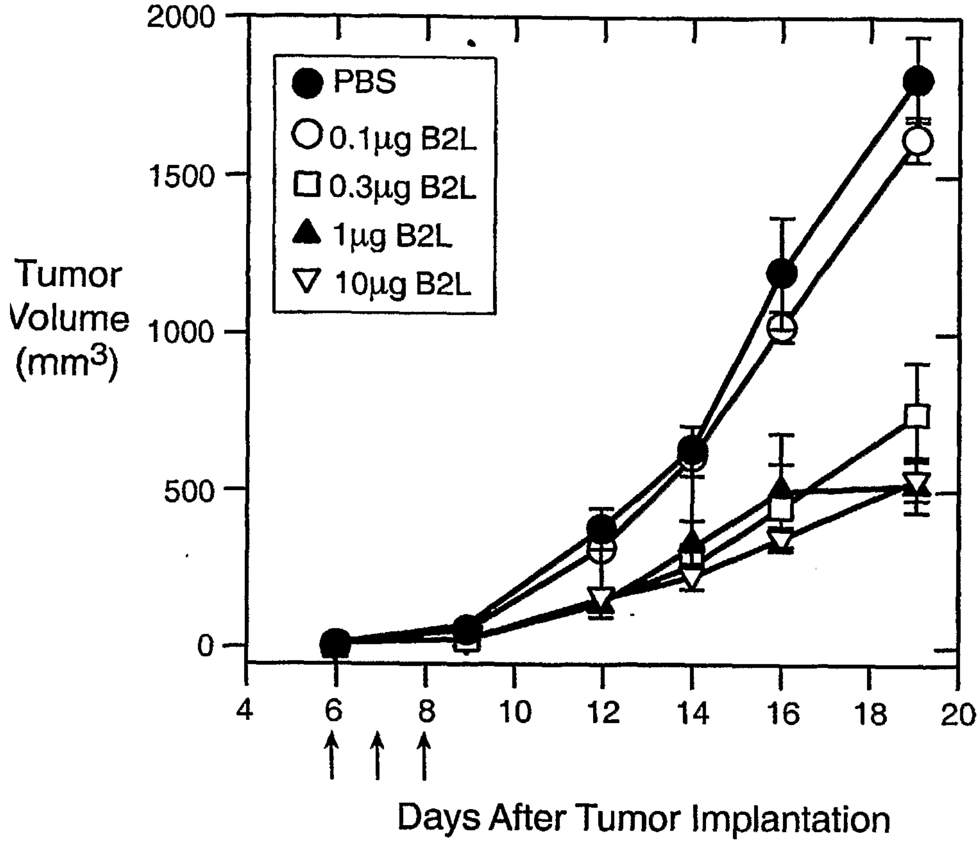

2000
1500
1000
500
0
Tumor
Volume
(mm3)
PBS
0.1μg B2L
0.3μg B2L
1μg B2L
10μg B2L
4
6
8
10
12
14
16
18
20
Days After Tumor Implantation

FIG._8

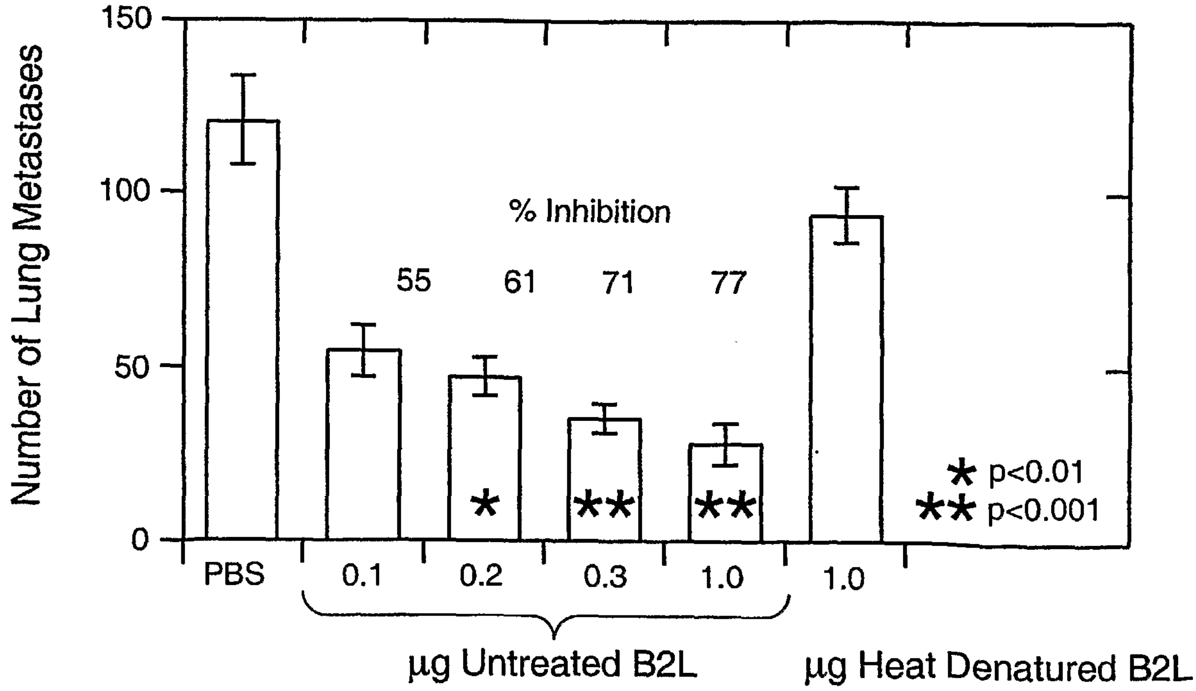

150
100
50
0
Number of Lung Metastases
% Inhibition
55 61 71 77
* p<0.01
** p<0.001
PBS
0.1
0.2
0.3
1.0
1.0
μg Untreated B2L
μg Heat Denatured B2L

FIG._9

SEQUENCE LISTING

<110> Dubois-Stringfellow, Nathalie *et al.*

<120> Use of *Parapox* B2L Protein to Treat Cancer and Modify Immune Responses

<130> 002973.00092

<160> 4

<170> FastSEQ for Windows Version 4.0

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<211> 378
<212> PRT
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Thr Leu Asp Cys Phe Thr Ala Ile Ala Glu Ser Ala Lys Lys Phe Leu
        35   40   45
Tyr Ile Cys Ser Phe Cys Cys Asn Leu Ser Ser Thr Lys Glu Gly Val
    50   55   60
Asp Val Lys Asp Lys Leu Cys Thr Leu Ala Lys Glu Gly Val Asp Val
65   70   75   80
Thr Leu Leu Val Asp Val Gln Ser Lys Asp Lys Asp Ala Asp Glu Leu
                85   90   95
Arg Glu Ala Gly Val Asn Tyr Tyr Lys Val Lys Val Ser Thr Lys Glu
            100   105   110
Gly Val Gly Asn Leu Leu Gly Ser Phe Trp Leu Ser Asp Ala Gly His
        115   120   125
Trp Tyr Val Gly Ser Ala Ser Leu Thr Gly Gly Ser Val Ser Thr Ile
    130   135   140
Lys Asn Leu Gly Leu Tyr Ser Thr Asn Lys His Leu Ala Trp Asp Leu
145   150   155   160
Met Asn Arg Tyr Asn Thr Phe Tyr Ser Met Ile Val Glu Pro Lys Val
                165   170   175
Pro Phe Thr Arg Leu Cys Cys Ala Ile Val Thr Pro Thr Ala Thr Asn
            180   185   190
Phe His Leu Asp His Ser Gly Gly Gly Val Phe Phe Ser Asp Ser Pro
        195   200   205
Glu Arg Phe Leu Gly Phe Tyr Arg Thr Leu Asp Glu Asp Leu Val Leu
    210   215   220
His Arg Ile Glu Asn Ala Lys Asn Ser Ile Asp Leu Ser Leu Leu Ser
225   230   235   240
Met Val Pro Val Ile Lys His Ala Ser Ala Val Glu Tyr Trp Pro Gln
                245   250   255
Ile Ile Asp Ala Leu Leu Arg Ala Ala Ile Asn Arg Gly Val Arg Val
            260   265   270

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Arg Val Ile Ile Thr Glu Trp Lys Asn Ala Asp Pro Leu Ser Val Ser
        275                 280                 285
Ala Ala Arg Ser Leu Asp Asp Phe Gly Val Gly Ser Val Asp Met Ser
    290                 295                 300
Val Arg Lys Phe Val Val Pro Gly Arg Asp Asp Ala Ala Asn Asn Thr
305                 310                 315                 320
Lys Leu Leu Ile Val Asp Asp Thr Phe Ala His Leu Thr Val Ala Asn
                325                 330                 335
Leu Asp Gly Thr His Tyr Arg Tyr His Ala Phe Val Ser Val Asn Ala
            340                 345                 350
Glu Lys Gly Asp Ile Val Lys Asp Leu Ser Ala Val Phe Glu Arg Asp
        355                 360                 365
Trp Arg Ser Glu Phe Cys Lys Pro Ile Asn
    370                 375
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<211> 1557
<212> DNA
<213> Parapox ovis strain NZ2

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caggtccttg acgatgtcgc ccttctcggc gttcacgctc acgaaggcgt ggtagcggta     120
gtgcgtgccg tcgaggttgg cgaccgtgag gtgcgcgaag gtgtcgtcca cgatgagcag     180
cttagtgttg ttcgcggcgt cgtcccggcc gggtaccacg aacttgcgca cggacatgtc     240
cacgctgccg acgccaaagt cgtcgaggct gcgcgcggcc gagaccgaaa gcgggtccgc     300
gttcttccac tcggtaatga tcacgcgcac gcgcacgccg cggttgatgg ccgcgcgcag     360
cagcgcgtca atgatctgcg gccagtactc cacggcgctg gcgtgcttga tcaccggcac     420
catcgagagc agcgagaggt cgatgctgtt cttggcgttc tcgatgcggt gcagcacgag     480
gtcctcgtcg agcgtgcggt agaagcctag gaagcgctcc ggcgagtccg agaagaatac     540
gccgcccccg gagtggtcga ggtggaagtt cgtggccgtg ggcgtgacga tggcgcagca     600
gagccgcgtg aacggcacct tcggctccac gatcatggag tagaaggtgt tgtagcggtt     660
catgaggtcc caggccaggt gcttgttggt ggagtagagc ccgaggttct tgatggtgga     720
cacggacccg cccgtgagcg aggcgcttcc cacgtaccag tgcccggcgt ccgagagcca     780
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Phe His Leu Asn His Ser Gly Gly Gly Val Phe Phe Ser Asp Ser Pro
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His Arg Ile Glu Asn Ala Lys Asn Ser Ile Asp Leu Ser Leu Leu Ser
225 230 235 240
Met Val Pro Val Ile Lys His Ala Gly Ala Val Glu Tyr Trp Pro Arg
245 250 255
Ile Ile Asp Ala Leu Leu Arg Val Ala Ile Asn Arg Gly Val Arg Val
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Arg Val Ile Ile Thr Glu Trp Lys Asn Ala Asp Pro Leu Ser Val Ser
275 280 285
Ala Ala Arg Ser Leu Asp Asp Phe Gly Val Gly Ser Val Asp Met Ser
290 295 300
Val Arg Lys Phe Val Val Pro Gly Arg Asp Asp Ala Ala Asn Asn Thr
305 310 315 320
Lys Leu Leu Ile Val Asp Asp Thr Phe Ala His Leu Thr Val Ala Asn
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Leu Asp Gly Thr His Tyr Arg Tyr His Ala Phe Val Ser Val Asn Ala
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专利名称(译)	使用parapox b2l蛋白来治疗癌症和改变免疫反应		
公开(公告)号	EP1499355A2	公开(公告)日	2005-01-26
申请号	EP2002789997	申请日	2002-12-06
[标]申请(专利权)人(译)	拜尔药品公司		
申请(专利权)人(译)	拜耳制药股份有限公司		
当前申请(专利权)人(译)	拜耳制药股份有限公司		
[标]发明人	CASSELL DELANIE TEPPER JEFFREY S SAMUELS ISA DUBOIS STRINGFELLOW NATHALIE		
发明人	CASSELL, DELANIE TEPPER, JEFFREY, S. SAMUELS, ISA DUBOIS-STRINGFELLOW, NATHALIE		
IPC分类号	A61K39/275 A61K39/39 A61K48/00 C07K14/065 C12Q1/02 G01N33/569 A61K45/00 G01N33/53 G01N33/566		
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其他公开文献	EP1499355A4		
外部链接	Espacenet		

摘要(译)

称为“B2L”的纯化的副痘病毒包膜蛋白可用作单一治疗剂。 B2L蛋白也可用于筛选方法，以鉴定用于调节受试者对B2L蛋白的免疫应答的潜在治疗剂。