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Genetic adjuvants for immunotherapy

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(54) **GENETIC ADJUVANTS FOR
IMMUNOTHERAPY**

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patent is extended or adjusted under 35
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This patent is subject to a terminal dis-
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See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,414,150	A	11/1983	Goeddel
4,456,748	A	6/1984	Goeddel
4,678,751	A	7/1987	Goeddel
4,695,623	A	9/1987	Stabinsky
4,897,471	A	1/1990	Stabinsky
5,571,515	A	11/1996	Scott et al.
5,731,490	A	3/1998	Mak et al.
5,744,166	A	4/1998	Illum
5,766,864	A	6/1998	Uno et al.
5,770,191	A	6/1998	Johnson et al.
5,831,062	A	11/1998	Taylor et al.
5,853,755	A	12/1998	Foldvari
5,861,300	A	1/1999	Silverman et al.
5,866,787	A	2/1999	Silverman et al.
6,120,762	A	9/2000	Johnson et al.
6,121,247	A	9/2000	Huang et al.
6,168,923	B1	1/2001	Scott et al.
6,210,663	B1	4/2001	Ertl
6,218,180	B1	4/2001	Kurtzman et al.
6,379,897	B1	4/2002	Weidenhammer et al.
6,399,322	B1	6/2002	Glimcher et al.
6,429,025	B1	8/2002	Parce et al.
6,475,995	B1	11/2002	Roy et al.
6,489,306	B2	12/2002	Mohapatra et al.
6,653,124	B1	11/2003	Freeman
6,693,086	B1	2/2004	Dow et al.

6,710,035	B2	3/2004	Felgner et al.
6,803,238	B1	10/2004	Eggers
6,811,788	B2	11/2004	Yu
7,029,838	B2	4/2006	Williams et al.
7,041,246	B2	5/2006	Fillmore et al.
7,052,685	B1	5/2006	Rook
7,052,829	B2	5/2006	Williams et al.
7,081,954	B2	7/2006	Sandstrom
7,267,958	B2	9/2007	Dordick et al.
7,354,908	B2	4/2008	Mohapatra et al.
2001/0006951	A1	7/2001	Mohapatra et al.
2002/0044919	A1	4/2002	Yu
2003/0068333	A1	4/2003	Mohapatra et al.
2003/0138404	A1	7/2003	Maroun
2003/0198624	A1	10/2003	Mohapatra et al.
2004/0009152	A1	1/2004	Mohapatra et al.
2004/0248323	A1	12/2004	Zhou et al.
2005/0025742	A1	2/2005	Engler et al.
2005/0054052	A1	3/2005	Carr et al.
2005/0054053	A1	3/2005	Aguinaldo et al.
2005/0084478	A1	4/2005	Liu et al.
2005/0266093	A1	12/2005	Mohapatra
2005/0272650	A1	12/2005	Mohapatra

(Continued)

FOREIGN PATENT DOCUMENTS

EP	343388	A2	11/1989
WO	WO 90/09780	A1	9/1990

(Continued)

OTHER PUBLICATIONS

Kumar et al. J Allergy Clin Immunol 108:402-408, Aug. 2001.*
Li et al. J Immunol 157:3216-3219, 1996.*
Hogan et al. Eur. J. Immunol. 28:413-423, 1998.*
AF101062 (<http://www.ncbi.nlm.nih.gov/nuccore/4323578>, see
printout dated Jan. 27, 2010 pp. 1-2).*
V00543 (<http://www.ncbi.nlm.nih.gov/nuccore/32722>, see printout
dated Jan. 27, 2010 pp. 1-3).*
Gould and Auchincloss. Immunology Today 20(2):77-82, 1999.*
Azimzadeh et al. Hematology and Cell Therapy 38(4):331-343,
1997.*
"Gene" from The American Heritage Dictionary of the English Lan-
guage, 4th Ed. (online), 2000 (retrieved on Jun. 15, 2006). Retrieved
from the Internet: <URL: <http://dictionary.reference.com/browse/gene>>, pp. 1-3.

(Continued)

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(57) **ABSTRACT**

The present invention pertains to methods and pharmaceu-
tical compositions for modulating an immune response. The
method of the present invention involves administration of an
effective amount of nucleic acid molecules encoding inter-
leukin-12 (IL-12), interferon-gamma (IFN-γ), or a combina-
tion thereof, to a patient in need of such treatment. The phar-
maceutical compositions of the invention contain nucleic acid
molecules encoding IL-12 and/or IFN-γ and an operably-
linked promoter sequence. In another aspect, the present
invention concerns expression vectors containing a nucle-
otide sequence encoding IL-12 and IFN-γ, and an operably-
linked promoter sequence. In another aspect, the present
invention concerns cells genetically modified with a nucle-
otide sequence encoding IL-12 and IFN-γ.

7 Claims, 5 Drawing Sheets
(2 of 5 Drawing Sheet(s) Filed in Color)

(56)

References Cited

U.S. PATENT DOCUMENTS

2005/0287118 A1 12/2005 Tian et al.
 2006/0276382 A1 12/2006 Mohapatra
 2007/0116767 A1 5/2007 Mohapatra
 2011/0052558 A1 3/2011 Mohapatra et al.

FOREIGN PATENT DOCUMENTS

WO WO 96/39806 A1 12/1996
 WO WO 98/02457 A1 1/1998
 WO WO 99/19496 A1 4/1999
 WO WO 01/22970 A1 4/2001
 WO WO 02/06343 1/2002
 WO WO 02/081638 A2 10/2002
 WO WO 03/028759 A1 4/2003
 WO WO 03/074561 A1 9/2003
 WO WO 03/089003 A1 10/2003
 WO WO 03/092618 A2 11/2003
 WO WO 2004/022003 A3 3/2004
 WO WO 2004/074314 A2 8/2004
 WO WO 2005/094420 A3 10/2005
 WO WO 2005/105136 A1 11/2005

OTHER PUBLICATIONS

"Immunocytokines—Local Stimulation of the Immune System", 2002, www.merck.at/produkte/immunozytokine.pdf, pp. 1-2.
 "Respiratory tract" from Wikipedia (online), 2006 (retrieved on Jun. 15, 2006). Retrieved from the Internet: <URL: http://en.wikipedia.org/wiki/Respiratory_tract>, pp. 1-2.
 "Timolol Maleate" from RxList The Internet Drug Index (online), 2006 (retrieved on Jun. 15, 2006). Retrieved from the Internet: <URL: http://www.rxlist.com/cgi/generic3/timololgfs_wcp.htm>, pp. 1-4.
 Adachi, Y. et al. "Amplitude and Frequency Modulation of Metabolic Signals in Leukocytes: Synergistic Role of IFN- γ in IL-6- and IL-2-Mediated Cell Activation" *The Journal of Immunology*, 1999, 163:4367-4374.
 Antoniou, K.M. et al. "Interferons and Their Application in the Diseases of the Lung" *Chest*, Jan. 2003, 123(1):209-216.
 Barnes, P.J. et al. "Cytokine-directed therapies for asthma" *J Allergy Clin Immunol*, 2001, 108(2):S72-S76.
 Behera, A.K. et al. "Adenovirus-Mediated Interferon γ Gene Therapy for Allergic Asthma: Involvement of Interleukin 12 and STAT4 Signaling" *Human Gene Therapy*, Sep. 2002, 13:1697-1709.
 Behera, A.K. et al. "2'-5' Oligoadenylate Synthetase Plays a Critical Role in Interferon- γ Inhibition of Respiratory Syncytial Virus Infection of Human Epithelial Cells" *The Journal of Biological Chemistry*, Jul. 2002, 277(28):25601-25608.
 Boraschi, D. et al. "Interferons inhibit LTC4 production in murine macrophages" *The Journal of Immunology*, Jun. 15, 1987, 138:4341-4346.
 Byrnes, A.A. et al. "Type I interferons and IL-12: convergence and cross-regulation among mediators of cellular immunity" *Eur. J. Immunol.*, 2001, 31:2026-2034.
 Carroll, M.W. et al. "Construction and Characterization of a Triple-Recombinant Vaccinia Virus Encoding B7-1, Interleukin 12, and a Model Tumor Antigen" *J Natl Cancer Inst*, 1998, 90:1881-1887.
 Cevc, G. et al. "Ultraflexible vesicles, Transfersomes, have an extremely low pore penetration and transport therapeutic amounts of insulin across the intact mammalian skin" *Biochimica et Biophysica Acta*, 1998, 1368:201-215.
 Chen, Q. et al. "Development of Th-1-type immune responses requires the type I cytokine receptor TCCR" *Nature*, 2000, 407:916-920.
 Cohen, J. "IL-12 deaths: explanation and a puzzle" *Science*, 1995, 270:908.
 Collins, P.L. et al. "Production of infectious human respiratory syncytial virus from cloned cDNA confirms an essential role for the transcription elongation factor from the 5' proximal open reading frame of the M2 mRNA in gene expression and provides a capability for vaccine development" *Proc Natl Acad Sci USA*, Dec. 1995, 92:11563-11567.

Connors, M. et al. "Respiratory Syncytial Virus (RSV) F, G, M2 (22K), and N Proteins Each Induce Resistance to RSV Challenge, but Resistance Induced by M2 and N Proteins Is Relatively Short-Lived" *Journal of Virology*, Mar. 1991, 65(3):1634-1637.
 Daines, M.O. and Hershey, G. "A novel mechanism by which interferon- γ can regulate interleukin (IL-13) responses" *J. Biol. Chem.*, 2002, 277(12):10387-10393.
 Deng, J.C. et al. "Transient transgenic expression of gamma interferon promotes *Legionella pneumophila* clearance in immunocompetent hosts" *Infection and Immunity*, 2001, 69(10):6382-6390.
 Dobson, J. "Gene therapy progress and prospects: magnetic nanoparticle-based gene delivery" *Gene Therapy*, 2006, 13:283-287.
 Domachowske, J.B. et al. "Respiratory Syncytial Virus Infection: Immune Response, Immunopathogenesis, and Treatment" *Clinical Microbiology Reviews*, Apr. 1999, 12(2):298-309.
 Dow, S. et al. "Systemic and local interferon γ gene delivery to the lungs for treatment of allergen-induced airway hyperresponsiveness in mice" *Human Gene Therapy*, Aug. 1999, 10:1905-1914.
 Flotte, T.R. and Laube, B.L. "Gene therapy in cystic fibrosis" *CHEST*, 2001, 120:124S-131S.
 Foldvari, M. and Moreland, A. "Clinical observations with topical liposome-encapsulated interferon alpha for the treatment of genital papillomavirus infections" *J. Liposome Res.*, 1997, 7:115-126.
 Foldvari, M. et al. "Liposome encapsulated prostaglandin E1 in erectile dysfunction: Correlation between in vitro delivery through foreskin and efficacy in patients" *Urology*, 1998, 52(5):838-843.
 Ford, J.G. et al. "IL-13 and IFN- γ : interactions in lung inflammation" *J. Immunol.*, 2001, 167:1769-1777.
 Gavett, S.H. et al. "Interleukin 12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and Th2 cytokine expression in mice" *J. Exp. Med.*, 1995, 182:1527-1536.
 Gill, D.R. et al. "The development of gene therapy for diseases of the lung" *Cell. Mol. Life Sci.* 2004, 61(3):355-368.
 Goeddel, D.V. et al. "The structure of eight distinct cloned human leukocyte interferon cDNAs" *Nature*, Mar. 1981, 290:20-26.
 Gray, P.W. et al. "Cloning and expression of murine immune interferon cDNA" *Proc Natl Acad Sci USA*, Oct. 1983, 80:5842-5846.
 Gurujeyalakshmi, G. and Giri, S.N. "Molecular mechanisms of antifibrotic effect of interferon gamma in bleomycin-mouse model of lung fibrosis: downregulation of TGF- β and procollagen I and III gene expression" *Exp. Lung Res.*, 1995, 21:791-808.
 Gutterman, J.U. "Cytokine therapeutics: Lessons from interferon α " *Proc. Natl. Acad. Sci. USA*, 1994, 91:1198-1205.
 De Jong, R. et al. "IL-2 and IL-12 Act in Synergy to Overcome Antigen-Specific T Cell Unresponsiveness in Mycobacterial Disease" *The Journal of Immunology*, 1997, 159:786-793.
 Gautam, A. et al. "Delivery systems for Pulmonary Gene Therapy" *Am J Respir Med*, 2002, 1(1):35-46.
 Grohmann, U. et al. "Positive Regulatory Role of IL-12 in Macrophages and Modulation by IFN- γ " *The Journal of Immunology*, 2001, 167:221-227.
 Bradley, L.M. et al. "A Direct Role for IFN-gamma in Regulation of Th1 Cell Development" *The Journal of Immunology*, 1996, 157:1350-1580.
 Genbank Accession No. B38957 (Dec. 1, 2000), pp. 1-2.
 Genbank Accession No. X13274 (Nov. 15, 1994), p. 1.
 Hansen, G. et al. "Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyperreactivity but cause severe airway inflammation" *J. Clin. Invest.*, 1999, 103:175-183.
 Hansen, G. et al. "CD4⁺T helper cells engineered to produce latent TGF- β 1 reverse allergen-induced airway hyperreactivity and inflammation" *J. Clin. Invest.*, 2000, 105:61-70.
 Hasbold, J. et al. "Integrating signals from IFN- γ and IL-4 by B cells: positive and negative effects on CD40 ligand-induced proliferation, survival, and division-linked isotype switching to IgG1, IgE, and IgG2a" *J. Immunol.*, 1999, 163:4175-4181.
 Hasko, G. and Szabo, C. "IL-12 as a therapeutic target for pharmacological modulation in immune-mediated and inflammatory diseases: regulation of T helper 1/T helper 2 responses" *Brit. J. Pharm.*, 1999, 127:1295-1304.
 He, T.-C. et al. "A simplified system for generating recombinant adenoviruses" *Proc. Natl. Acad. Sci. USA*, 1998, 95:2509-2514.

(56)

References Cited

OTHER PUBLICATIONS

- Hellerman, G.R. et al. "Genetic therapy: on the brink of a new future" *Genetic Vaccines and Therapy*, 2003, 1(1):1-2.
- Henco, K. et al. "Structural Relationship of Human Interferon Alpha Genes and Pseudogenes" *J Mol Biol*, 1985, 185:227-260.
- Hofstra, C. et al. "Differential effects of endogenous and exogenous interferon- γ on immunoglobulin E, cellular infiltration, and airway responsiveness in a murine model of allergic asthma" *Am. J. Respir. Cell Mol. Biol.*, 1998, 19:826-835.
- Hsu, S.C. et al. "Synergistic effect of immunization with a peptide cocktail inducing antibody, helper and cytotoxic T-cell responses on protection against respiratory syncytial virus" *Journal of General Virology*, 1999, 80:1401-1405.
- Huang, T.-J. et al. "Allergen-specific Th1 cells counteract effluent Th2 cell-dependent bronchial hyperresponsiveness and eosinophilic inflammation partly via IFN- γ " *J. Immunol.*, 2001, 166:207-217.
- Jameton, R. et al. "IL-12 Possibilities" *Science*, Sep. 15, 1995, 269(5230):1498-1499.
- Kai, E. et al. "A Method for Oral DNA Delivery with N-Acetylated Chitosan" *Pharmaceutical Research*, May 2004, 21(5):838-843.
- Kaplan, M.H. et al. "Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice" *Nature*, 1996, 382:174-177.
- Kaplan, M.H. et al. "A signal transducer and activator of transcription (Stat)4-independent pathway for the development of T helper type 1 cells" *J. Exp. Med.*, 1998, 188(6):1191-1196.
- Kay, M.A. et al. "Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics" *Nature Med.*, 2001, 7:33-40.
- Kinsey, B. et al. "Non-viral gene delivery to the lungs" *Current Gene Therapy*, 2005, 5:181-194.
- Kirkwood, J.M. et al. "Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: The Eastern Cooperative Oncology Group trial EST 1684" *J. Clin. Oncology*, 1996, 14(1):7-17.
- Kitagawa, M. et al. "Interferon- γ enhances interleukin 12 production in rheumatoid synovial cells via CD40-CD154 dependent and independent pathways" *J. Rheumatology*, 2001, 28:1764-1771.
- Kreiss, P. et al. "Plasmid DNA size does not affect the physicochemical properties of lipoplexes but modulates gene transfer efficiency" *Nucleic Acids Research*, 1999, 27(19):3792-3798.
- Kumar, M. et al. "Intranasal IFN- γ gene transfer protects BALB/c mice against respiratory syncytial virus infection" *Vaccine*, 2000, 18:558-567.
- Kumar, M. et al. "Role of mucosal IFN- γ gene transfer on allergic sensitization and RSV infection" *J. Allergy Clin. Immunol.*, 2002, 109:S4, abstract No. 78.
- Kumar, M. et al. "Intranasal Gene Transfer by Chitosan-DNA Nanospheres Protects BALB/c Mice Against Acute Respiratory Syncytial Virus Infection" *Human Gene Therapy*, Aug. 2002, 13(12):1415-1425.
- Kumar, M. et al. "Chitosan IFN- γ -pDNA Nanoparticle (CIN) Therapy for Allergic Asthma" *Genetic Vaccines and Therapy*, 2003, 1(3):1-10.
- Kumar, M.N.V.R. et al. "Nanoparticle-mediated gene delivery: state of the art" *Expert Opin. Biol. Ther.*, 2004, 4(8):1213-1224.
- Lack, G. et al. "Nebulized but not parenteral IFN- γ decreases IgE production and normalizes airways function in a murine model of allergen sensitization" *J. Immunol.*, 1994, 152:2546-2554.
- Lengyel, P. et al. "Tumor-suppressor genes: News about the interferon connection" *Proc Natl Acad Sci USA*, Jul. 1993, 90:5893-5895.
- Leong, K.W. et al. "DNA-polycation nanospheres as non-viral gene delivery vehicles" *J. Controlled Release*, 1998, 53:183-193.
- Li, X. et al. "Protection against Respiratory Syncytial Virus Infection by DNA Immunization" *J Exp Med*, Aug. 17, 1998, 188(4):681-688.
- Li, X. et al. "Plasmid DNA Encoding the Respiratory Syncytial Virus G Protein Is a Promising Vaccine Candidate" *Virology*, 2000, 269:54-65.
- Lighvani, A.A. et al. "T-bet is rapidly induced by interferon- γ in lymphoid and myeloid cells" *PNAS*, 2001, 98(26):15137-15142.
- Mao, H.-Q. et al. "Chitosan-DNA nanoparticles as gene carriers: synthesis, characterization and transfection efficiency" *J. Controlled Release*, 2001, 70(3):399-421.
- Marshall, E. "Gene Therapy's Growing Pains" *Science*, 1995, 269:1050-1055.
- Matsuse, H. et al. "Recurrent Respiratory Syncytial Virus Infections in Allergen-Sensitized Mice Lead to Persistent Airway Inflammation and Hyperresponsiveness" *The Journal of Immunology*, 2000, 164:6583-6592.
- Minshall, E.M. et al. "Eosinophil-associated TGF- β 1 mRNA expression and airways fibrosis in bronchial asthma" *Am. J. Respir. Cell Mol. Biol.*, 1997, 17:326-333.
- Mohapatra, S. "Mucosal gene expression vaccine: a novel vaccine strategy for respiratory syncytial virus" *The Pediatric Infectious Disease Journal*, Feb. 2003, 22(2):S100-S104.
- Mohapatra, S.M. et al., poster materials presented at the American Academy of Allergy Asthma in Mar. 2002 and presented at the Immunology and American Thoracic Society in May 2002.
- Montgomery, D. et al. "DNA vaccines" *Pharmacol. Ther.*, 1997, 74:195-205.
- Morrison, D.F. and Murtaugh, M.P. "Adenovirus-mediated expression of interleukin-1 receptor antagonist in swine cells in vitro and in vivo" *Veterinary Immunol. and Immunopathology*, 2001, 78:71-81.
- Mosmann, T.R. and Coffman, R.L. "TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties" *Ann. Rev. Immunol.*, 1989, 7:145-173.
- Mullen, A.C. et al. "Role of T-bet in commitment of T_H1 cells before IL-12-dependent selection" *Science*, 2001, 292:1907-1909.
- Murray, H.W. "Current and future clinical applications of interferon-gamma in host antimicrobial defense" *Intensive Care Med*, 1996, 22(Suppl 4):S456-S461.
- Nakahira, M. et al. "Synergy of IL-12 and IL-18 for IFN- γ Gene Expression: IL-12-Induced STAT4 Contributes to IFN- γ Promoter Activation by Up-Regulating the Binding Activity of IL-18-Induced Activator Protein 1" *The Journal of Immunology*, 2002, 168:1146-1153.
- Nonaka, M. et al. "Induction of eotaxin production by interleukin-4, interleukin-13 and lipopolysaccharide by nasal fibroblasts" *Clin. Exp. Allergy*, 2004, 34:804-811.
- O'Donnell, M.A. et al. "Role of IL-12 in the Induction and Potentiation of IFN- γ in Response to *Bacillus Calmette-Guerin*" *The Journal of Immunology*, 1999, 163:4246-4252.
- O'Garra, A. and Arai, N. "The molecular basis of T helper 1 and T helper 2 cell differentiation" *Cell Biol.*, 2000, 10:542-550.
- Okubo, T. et al. "Administration of an IL-12-Encoding DNA Plasmid Prevents the Development of Chronic Graft-Versus-Host Disease (GVHD)" *The Journal of Immunology*, 1999, 162:4013-4017.
- Opdenakker, G. et al. "Interaction of interferon with other cytokines" *Experientia*, 1989, 45:513-520.
- Oppmann, B. et al. "Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12" *Immunity*, 2000, 13:715-725.
- Orkin, Report and recommendations of the panel to assess the NIH investment in research on gene therapy 1998, pp. 1-41.
- Park, K.H. et al. "Antiallergic Activity of a Disaccharide Isolated from *Sanguisorba officinalis*" *Phytotherapy Research*, 2004, 18:658-662.
- Park, S.-Y. et al. "IFN- γ enhances TRAIL-induced apoptosis through IRF-1" *Eur. J. Biochem.*, 2004, 271(21):4222-4228.
- Payne, L.G. et al. "Particle-mediated DNA vaccination of mice, monkeys and men: Looking beyond the dogma" *Current Opinion in Molecular Therapeutics*, 2002, 4:459-466.
- Perricone, M.A. et al. "Aerosol and Lobar Administration of a Recombinant Adenovirus to Individuals with Cystic Fibrosis. II. Transfection Efficiency in Airway Epithelium" *Human Gene Therapy*, 2001, 12:1383-1394.
- Pierkes, M. et al. "Decreases release of histamine and sulfidoleukotrienes by human peripheral blood leukocytes after wasp venom immunotherapy is partially due to induction of IL-10 and IFN- γ production of T cells" *J. Allergy Clin. Immunol.*, 1999, 103:326-332.

(56)

References Cited

OTHER PUBLICATIONS

- Pizzoferrato, E. et al. "Ectopic Expression of Interferon Regulatory Factor-1 Promotes Human Breast Cancer Cell Death and Results in Reduced Expression of Survivin" *Cancer Research*, Nov. 2004, 64:8381-8388.
- Pouton, C. and Seymour, L. "Key issues in non-viral gene delivery" *Adv. Drug Deliv. Rev.*, 2001, 46:187-203.
- Randolph, D.A. et al. "Modulation of airway inflammation by passive transfer of allergen-specific Th1 and Th2 cells in a mouse model of asthma" *J. Immunol.*, 1999, 162:2375-2383.
- Read, M. et al. "Barriers to gene delivery using synthetic vectors" *Adv. in Genetics*, 2005, 53:19-46.
- Rener, X. et al. "Construction and identification of a recombinant adenovirus which expresses human interferon- γ " *Chinese J. Biotech.*, 1997, 13:1-8.
- Robinson, D.S. et al. "Predominant T_{H2}-like bronchoalveolar T-lymphocyte population in atopic asthma" *N. Engl. J. Med.*, 1992, 326:298-304.
- Robinson, M. et al. "Predictive assessment of respiratory sensitizing potential of proteins in mice" in *Toxicology of Chemical Respiratory Hypersensitivity*, Dearman and Kimber, Eds., 1997, pp. 135-150.
- Roy, K. et al. "Oral gene delivery with chitosan? DNA nanoparticles generates immunologic protection in a murine model of peanut allergy" *Nature Medicine*, 1999, 5(4):387-391.
- Rudick, R.A. et al. "Management of multiple sclerosis" *J. Engl. J. Med.*, 1997, 337(22):1604-1611.
- Scheerlinck, J-P. "Genetic adjuvants for DNA vaccines" *Vaccine*, 2001, 19:2647-2656.
- Schwarze, J. et al. "Local treatment with IL-12 is an effective inhibitor of airway hyperresponsiveness and lung eosinophilia after airway challenge in sensitized mice" *J. Allergy Clin. Immunol.*, 1998, 102:86-93.
- Sethi, S.K. et al. "Interferon-gamma (IFN- γ) down-regulates the rhinovirus-induced expression of intercellular adhesion molecule-1 (ICAM-1) on human airway epithelial cells" *Clin. Exp. Immunol.*, 1997, 110:362-369.
- Short, S.M. et al. "Percutaneous absorption of biologically-active interferon-gamma in a human skin graft-nude mouse model" *Pharm. Res.*, 1996, 13:1020-1027.
- Simmons, C.P. et al. "Mucosal delivery of a respiratory syncytial virus CTL peptide with enterotoxin-based adjuvants elicits protective, immunopathogenic, and immunoregulatory antiviral CD8⁺ T cell responses" *J. Immun.*, 2001, 166:1106-1113.
- Somia, N. and Verma, I.M. "Gene therapy: Trials and tribulations" *Nature Rev.*, 2000, 1:91-99.
- Stallmach, A. et al. "An Interleukin 12 p40-IgG2b Fusion Protein Abrogates T Cell Mediated Inflammation: Anti-Inflammatory Activity in Crohn's Disease and Experimental Colitis In Vivo", *Gut*, 2004, pp. 339-345, vol. 53.
- Stampfli, M.R. et al. "GM-CSF transgene expression in the airway allows aerosolized ovalbumin to induce allergic sensitization in mice" *J. Clin. Invest.*, 1998, 102:1704-1714.
- Stirling, R.G. and Chung, K.F. "New immunological approaches and cytokine targets in asthma and allergy" *Eur. Respir. J.*, 2000, 16:1158-1174.
- Streuli, M. et al. "At Least Three Human Type a Interferons: Structure of a2" *Science*, Sep. 19, 1980, 209:1343-1347.
- Sur, S. et al. "Mucosal IL-12 inhibits airway reactivity to methacholine and respiratory failure in murine asthma" *Exp. Lung Res.*, 2000, 26:477-489.
- Tang, Y.W. et al. "Determinants and kinetics of cytokine expression patterns in lungs of vaccinated mice challenged with respiratory syncytial virus" *Vaccine*, 1997, 15(6/7):597-602.
- Tang, C. et al. "Th type 1-stimulating activity of lung macrophages inhibits Th2-mediated allergic airway inflammation by an IFN- γ -dependent mechanism" *J. Immunol.*, 2001, 166:1471-1481.
- Thierfelder, W.E. et al. "Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells" *Nature*, 1996, 382:171-174.
- Thivierge, M. et al. "IL-13 and IL-4 up-regulated cysteinyl leukotriene 1 receptor expression in human monocytes and macrophages" *J. Immunol.*, 2001, 167:2855-2860.
- Trinchieri, G. "Interleukin-12: a cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cells type 1 and cytotoxic lymphocytes" *Blood*, 1994, 84(12):4008-4027.
- Umetsu, D.T. and Dekruyff, R.H. "TH1 and TH2 CD4⁺ cells in human allergic diseases" *J. Allergy Clin. Immunol.*, 1997, 100(1):1-6.
- Upton, C. et al. "Encoding of a homolog of the IFN- γ receptor by myxoma virus" *Science*, 1992, 258:1369-1372.
- Van Den Hurk, S.V.D.L. et al. "Strategies for improved formulation and delivery of DNA vaccines to veterinary target species" *Immunological Reviews*, 2004, 199:113-125.
- Vandenbroeck, K. et al. "The conserved helix C region in the superfamily of interferon- γ /interleukin-10-related cytokines corresponds to a high-affinity binding site for the HSP70 chaperone DnaK" *J. Biol. Chem.*, 2002, 277:25668-25676.
- Verma, I.M. et al. "Gene Therapy—promises, problems and prospects" *Nature*, 1997, 389:239-242.
- Wahren, B. et al. "DNA Vaccines: An Overview" in *DNA Pharmaceuticals: Formulation and Delivery in Gene Therapy, DNA Vaccination and Immunotherapy*, 2005, Wiley-VCH Verlag GmbH & Co, pp. 1-6.
- Walter, D.M. et al. "IL-18 gene transfer by adenovirus prevents the development of and reverses established allergen-induced airway hyperactivity" *J. Immunol.*, 2001, 166:6392-6398.
- Wang, I-M. et al. "An IFN- γ -inducible transcription factor, IFN consensus sequence binding protein (ICSBP), stimulates IL-12 p40 expression in macrophages" *J. Immunol.*, 2000, 165:271-279.
- Wenner, C.A. et al. "Roles of IFN-gamma and IFN-alpha in IL-12-Induced T Helper Cell-1 Development" *The Journal of Immunology*, 1996, 156:1442-1447.
- Wright, P. et al. "Evaluation of a live, cold-passaged, temperature-sensitive, respiratory syncytial virus vaccine candidate in infancy" *J. Infect. Dis.*, 2000, 182:1331-1342.
- Wyatt, L.S. et al. "Priming and boosting immunity to respiratory syncytial virus by recombinant replication-defective vaccinia virus MVA" *Vaccine*, 2000, 18:392-397.
- Xiu, Q. et al. "Effect of intratracheally administered IL-12 recombinant adenovirus on ovalbumin induced bronchial hyperresponsiveness in mouse model" *Chin J Tuberc Respir Dis*, 2001, 24:298-301, abstract.
- Yang, X. "Imaging of Vascular Gene Therapy" *RSNA*, 2003, 228(1):36-49.
- Yoshida, M. et al. "Effect of interferon- γ on allergic airway responses in interferon- γ -deficient mice" *Am J Respir Crit Care Med*, 2002, 166:451-456.
- Zabner, J. et al. "Repeat Administration of an Adenovirus Vector Encoding Cystic Fibrosis Transmembrane Conductance Regulator to the Nasal Epithelium of Patients with Cystic Fibrosis" *J Clin Invest*, 1996, 97:1504-1511.
- Zhu, Z. et al. "Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation" *Science*, 2004, 304:1678-1682.
- Zoon, K.C. "Human interferons: Structure and function" *Interferon*, 1987, 9:1-12.
- Cincinnati Children's Hospital, "2006 Pediatric Health News Releases" <http://www.cincinnatichildrens.org/about/news/release/2006/3-gene-therapy.htm>, Mar. 31, 2006, 2 pages.
- Addison, C.L. et al. "The CXCL12 Chemokine, Monokine Induced by Interferon- γ , Inhibits Non-Small Cell Lung Carcinoma Tumor Growth and Metastasis" *Human Gene Therapy*, Jan. 20, 2000, 11:247-261.
- Altman, C.A. et al. "Respiratory Syncytial Virus in Patients with Congenital Heart Disease: A Contemporary Look at Epidemiology and Success of Preoperative Screening" *Pediatric Cardiology*, Sep. 2000, 21(5):433-438.
- Aral, C. et al. "Preparation and in vitro transfection efficiency of chitosan microspheres containing plasmid DNA:poly(L-lysine) complexes" *J Pharm Pharmaceut Sci*, Sep.-Dec. 2003, 6(3):321-326.

(56)

References Cited

OTHER PUBLICATIONS

- Arenberg, D.A. et al. "Improved survival in tumor-bearing SCID mice treated with interferon- γ -inducible protein 10 (IP-10/CXCL10)" *Cancer Immunology, Immunotherapy*, 2001, 50:533-538.
- Banerjee, R. et al. "Selective inhibition of hepatitis B virus and human immunodeficiency virus sequence-promoted gene expression by cotransfected poly(I):poly(C)" *Virology*, Nov. 1990, 179(1):410-415.
- Barnett, B.G. et al. "Targeted adenoviral vectors" *Biochimica et Biophysica Acta*, 2002, 1575:1-14.
- Behera, A.K. et al. "Mucosal 2'-5' Oligoadenylate Synthetase (2-5 AS) Gene Transfer Attenuates Respiratory Syncytial Virus Infection in a Murine Model" *J Allergy Clin Immunol*, Jan. 2002, 109(1):S43, abstract 79.
- Benavente, J. et al. "Indiscriminate degradation of RNAs in interferon-treated, vaccinia virus-infected mouse L cells" *Journal of Virology*, Sep. 1984, 51:866-871.
- Benech, P. et al. "Structure of two forms of the interferon-induced (2'-5') oligo A synthetase of human cells based on cDNAs and gene sequences" *The EMBO Journal*, 1985, 4(9):2249-2256.
- Bisbal, C. et al. "Cloning and Characterization of a RNase L Inhibitor: A New Component of the Interferon-Regulated 2-5A Pathway" *The Journal of Biological Chemistry*, Jun. 1995, 270(22):13308-13317.
- Bivas-Benita, M. et al. "Pulmonary delivery of chitosan-DNA nanoparticles enhances the immunogenicity of a DNA vaccine encoding HLA-A*0201-restricted T-cell epitopes of *Mycobacterium tuberculosis*" *Vaccine*, 2004, 22:1609-1615.
- Blanck, G. "Components of the IFN- γ Signaling Pathway in Tumorigenesis" *Archivum Immunologiae et Therapiae Experimentalis*, 2002, 50:151-158.
- Boccaccio, G.L. et al. "Non-coding plasmid DNA induces IFN- γ in vivo and suppresses autoimmune encephalomyelitis" *International Immunology*, Feb. 1999, 11(2):289-296.
- Boguniewicz, M. et al. "The effects of nebulized recombinant interferon- γ in asthmatic airways" *J Allergy Clin Immunol*, Jan. 1995, 95(1):133-135.
- Boguniewicz, M. et al. "Atopy, airway responsiveness, and genes" *Thorax*, 1996, 51(Supplement 2):S55-S59.
- Chada, S. et al. "Cytokine- and chemokine-based gene therapy for cancer" *Curr Opin Mol Ther.*, Oct. 2003, 5(5):463-474, abstract.
- Chebath, J. et al. "Four Different Forms of Interferon-induced 2',5'-Oligo(A) Synthetase Identified by Immunoblotting in Human Cells" *The Journal of Biological Chemistry*, Mar. 1987, 262(8):3852-3857.
- Chen, J.H. "Application of cationic polymer vector for gene delivery systems" Yao Xue Xue Bao, Apr. 2003, 38(4):316-20, abstract.
- Choudhary, S. et al. "Interferon Action against Human Parainfluenza Virus Type 3: Involvement of a Novel Antiviral Pathway in the Inhibition of Transcription" *Journal of Virology*, May 2001, 75(10):4823-4831.
- Chung, F. "Anti-inflammatory cytokines in asthma and allergy: interleukin-10, interleukin-12, interferon- γ " *Mediators of Inflammation*, 2001, 10:51-59.
- Chung, K.F. et al. "Cytokines in asthma" *Thorax*, Sep. 1999, 54:825-857.
- Coccia, E.M. et al. "Cells Resistant to Interferon- β Respond to Interferon- γ via the Stat1-IRF-1 Pathway" *Virology*, 1995, 211:113-122.
- Cohen, B. et al. "Enhancer-like interferon responsive sequences of the human and murine (2'-5') oligoadenylate synthetase gene promoters" *The EMBO Journal*, 1988, 7(5):1411-1419.
- Cohen, A.D. et al. "Modulating the immune response to genetic immunization" *FASEB J*, 1998, 12:1611-1626.
- Cohn, L. et al. "T Helper 1 Cells and Interferon γ Regulate Allergic Airway Inflammation and Mucus Production" *Journal of Experimental Medicine*, Nov. 1999, 190(9):1309-1317.
- Corrias, M.V. et al. "Induction of 2,5 OAS Gene Expression and Activity Is Not Sufficient for IFN- γ -Induced Neuroblastoma Cell Differentiation" *Int J Cancer*, 1995, 62:223-229.
- David, S. et al. "2'-5'-Oligoadenylate Synthetase Gene Expression in Normal and Murine Sarcoma Virus-Transformed NIH 3T3 Cells" *Journal of Virology*, Mar. 1989, 63(3):1116-1122.
- D'Cunha, J. et al. "In Vitro and In Vivo Secretion of Human ISG15, an IFN-Induced Immunomodulatory Cytokine" *The Journal of Immunology*, Nov. 1996, 157:4100-4108.
- D'Cunha, J. et al. "Immunoregulatory properties of ISG15, an interferon-induced cytokine" *Proc. Natl Acad. Sci. USA*, Jan. 1996, 93:211-215.
- Dorsey, R. et al. "Immunotherapy with Interleukin-10 Depends on the CXC Chemokines Inducible Protein-10 and Monokine Induced by IFN- γ " *Cancer Research*, May 2002, 62:2606-2610.
- Englebienne, P. et al. "RNase L in Health and Disease: What Did We Learn Recently" *Journal of Chronic Fatigue Syndrome*, 2003, 11(2):97-109.
- Engelhardt, O.G. et al. "Interferon-Induced Antiviral Mx1 GTPase Is Associated with Components of the SUMO-1 System and Promyelocytic Leukemia Protein Nuclear Bodies" *Experimental Cell Research*, 2001, 271:286-295.
- Eskildsen, S. et al. "Characterization of the 2'-5'-oligoadenylate synthetase ubiquitin-like family" *Nucleic Acids Research*, 2003, 31(12):3166-3173.
- Esteban, M. et al. "Identification by Electron Microscopy of the Maturation Steps in Vaccinia Virus Morphogenesis Inhibited by the Interferon-Induced Enzymes, Protein Kinase (PKR), 2-5A Synthetase, and Nitric Oxide Synthase (iNOS)" *Journal of Interferon and Cytokine Research*, 2000, 20:867-877.
- Ghosh, A. et al. "Enzymatic Activity of 2'-5'-Oligoadenylate Synthetase Is Impaired by Specific Mutations that Affect Oligomerization of the Protein" *The Journal of Biological Chemistry*, Dec. 26, 1997, 272(52):33220-33226.
- Ghosh, A. et al. "Cell Growth Regulatory and Antiviral Effects of the P69 Isozyme of 2-5 (A) Synthetase" *Virology*, 2000, 266:319-328.
- Goswami, B.B. et al. "Degradation of rRNA in Interferon-treated Vaccinia Virus-infected Cells" *The Journal of Biological Chemistry*, Feb. 10, 1984, 259(3):1371-1374.
- Grander, D. et al. "In Vivo Induction of the Interferon-Stimulated Protein 2'-5'-Oligoadenylate Synthetase in Tumor and Peripheral Blood Cells During IFN- α Treatment of Metastatic Melanoma" *Journal of Interferon and Cytokine Research*, 1998, 18:691-695.
- Groothuis, J.R. et al. "Respiratory Syncytial Virus Infection in Children With Bronchopulmonary Dysplasia" *Pediatrics*, 1988, 82:199-203.
- Guille, M.J. et al. "Functional differences in the promoters of the interferon-inducible (2'-5')A oligoadenylate synthetase and 6-16 genes in interferon-resistant Daudi Cells" *European Journal of Biochemistry*, Jan. 1994, 219:547-553.
- Hartmann, R. et al. "Activation of 2'-5' Oligoadenylate Synthetase by Single-stranded and Double-stranded RNA Aptamers" *The Journal of Biological Chemistry*, Feb. 1998, 273(6):3236-3246.
- Hassel, B.A. et al. "A dominant negative mutant of 2-5A-dependent RNase suppresses antiproliferative and antiviral effects of interferon" *The EMBO Journal*, 1993, 12(8):3297-3304.
- Hertel, L. et al. "The retinoblastoma protein is an essential mediator that links the interferon-inducible 204 gene to cell-cycle regulation" *Oncogene*, Jul. 2000, 19(32):3598-3608.
- Hillinger, S. et al. "EBV-Induced Molecule 1 Ligand Chemokine (ELC/CCL 19) Promotes IFN- γ -Dependent Antitumor Responses in a Lung Cancer Model" *The Journal of Immunology*, 2003, 171:6457-6465.
- Huang, T.J. et al. "Inhibitory effects of endogenous and exogenous interferon- γ on bronchial hyperresponsiveness, allergic inflammation and T-helper 2 cytokines in Brown-Norway rats" *Immunology*, Oct. 1999, 98:280-288.
- Huang, H. et al. "Synergistic Effect of Lymphotoxin and Interferon gamma-Inducible Protein-10 Transgene Expression in T-Cell Localization and Adoptive T-Cell Therapy of Tumors" *Int. J. Cancer*, 2004, 109:817-825.
- Jakschies, D. et al. "Correlation of the Antiproliferative Effect and the Mx-Homologous Protein Induction by IFN in Patients with Malignant Melanoma" *J Invest Dermatol*, 1990, 95:238S-241S.

(56)

References Cited

OTHER PUBLICATIONS

- Jakschies, D. et al. "Emergence and decay of the human Mx homolog in cancer patients during and after interferon-alpha therapy" *J Biol Response Mod.*, Jun. 1990, 9(3):305-312, abstract.
- Jungwirth, C. et al. "Chicken interferon consensus sequence-binding protein (ICSBP) and interferon regulatory factor (IRF) 1 genes reveal evolutionary conservation in the IRF gene family" *Proc. Natl. Acad. Sci. USA*, Apr. 1995, 92:3105-3109.
- Justesen, J. et al. "Gene structure and function of the 2'-5'-oligoadenylate synthetase family" *CMLS, Cellular and Molecular Life Sciences*, 2000, 57:1593-1612.
- Justesen, J. et al. "Spectrophotometric Pyrophosphate Assay of 2',5'-Oligoadenylate Synthetase" *Analytical Biochemistry*, 1992, 207:90-93.
- Kim, T.H. et al. "Efficient gene delivery by urocanic acid-modified chitosan" *Journal of Controlled Release*, 2003, 93:389-402.
- Kim, P.K.M. et al. "IRF-1 expression induces apoptosis and inhibits tumor growth in mouse mammary cancer cells in vitro and in vivo" *Oncogene*, 2004, 23:1125-1135.
- Kumar, R. et al. "Studies on the Role of the 2'-5'-Oligoadenylate Synthetase-RNase L Pathway in Beta Interferon-Mediated Inhibition of Encephalomyocarditis Virus Replication" *Journal of Virology*, Sep. 1988, 62(9):3175-3181.
- Kumar, R. et al. "Growth inhibition of human acute promyelocytic leukemia NB-4 cells by interferons and all-trans retinoic acid: transmodulation of inducible gene expression pathways" *Anticancer Research*, Mar.-Apr. 1995, 15(2):353-360, abstract.
- Li, X.L. et al. "RNase L Mediates the Antiviral Effect of Interferon through a Selective Reduction in Viral RNA during Encephalomyocarditis Virus Infection" *Journal of Virology*, Apr. 1998, 72(4):2752-2759.
- Liberati, A.M. et al. "Interferon-alpha-Induced Biologic Modifications in Patients with Chronic Myelogenous Leukemia" *Journal of Interferon Research*, Dec. 1994, 14:349-355.
- Lotem, J. et al. "Interferon-gamma inhibits apoptosis induced by wild-type p53, cytotoxic anti-cancer agents and viability factor deprivation in myeloid cells" *Leukemia*, 1995, 9:685-692.
- Luster, A.D. et al. "IP-10, a C-X-C-Chemokine, Elicits a Potent Thymus-dependent Antitumor Response In Vivo" *The Journal of Experimental Medicine*, Sep. 1993, 178(3):1057-1065.
- Maitra, R.K. et al. "Regulation of Human Immunodeficiency Virus Replication by 2',5'-Oligoadenylate-Dependent RNase L" *Journal of Virology*, Feb. 1998, 72(2):1146-1152.
- Matsuse, H. et al. "Differential cytokine mRNA expression in *Dermatophagoides farinae* allergen-sensitized and respiratory syncytial virus-infected mice" *Microbes and Infection*, 2000, 2:753-759.
- Melaine, N. et al. "Production of the antiviral proteins 2'5'oligoadenylate synthetase, PKR and Mx in interstitial cells and spermatogonia" *Journal of Reproductive Immunology*, 2003, 59:53-60.
- Millar, B.C. et al. "2',5'- Oligoadenylate synthetase levels in patients with multiple myeloma receiving maintenance therapy with interferon $\alpha 2b$ do not correlate with clinical response" *British Journal of Cancer*, 1995, 72:1525-1530.
- Miyamoto, M. et al. "Regulated Expression of a Gene Encoding a Nuclear Factor, IRF-1, That Specifically Binds to IFN- β Gene Regulatory Elements" *Cell*, Sep. 9, 1988, 54:903-913.
- Mohapatra, S. Poster materials presented at International RSV Conference on Jun. 22, 2002, Washington, D.C. (9 pages, including abstract and Figures 1-4 with captions).
- Monahan, P.E. et al. "Adeno-associated virus vectors for gene therapy: more pros than cons?" *Molecular Medicine Today*, Nov. 2000, 6:433-440.
- Morgan, R.A. et al. "Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes" *Science*, Oct. 6, 2006, 314(5796):126-129.
- University of South Florida, "Novel Gene Therapy Effectively Reduces Asthma Symptoms in Mice" released Oct. 31, 2003, 7:00 a.m., <http://www.newswise.com/articles/view/501702/>, pp. 1-2.
- Nilsen, T.W. et al. "Double-stranded RNA Causes Synthesis of 2',5'-Oligo(A) and Degradation of Messenger RNA in Interferon-treated Cells" *The Journal of Biological Chemistry*, Aug. 1981, 256(15):7806-7811.
- Nilsen, T.W. et al. "Synthesis of (2'-5')Oligoadenylate and Activation of an Endoribonuclease in Interferon-Treated HeLa Cells Infected with Reovirus" *Journal of Virology*, Jun. 1982, 42(3):1039-1045.
- Office Action mailed Sep. 19, 2007 in U.S. Appl. No. 11/117,169, filed Apr. 27, 2005.
- Onari, Y. et al. "IL-12p40 is essential for the down-regulation of airway hyperresponsiveness in a mouse model of bronchial asthma with prolonged antigen exposure" *Clinical and Experimental Allergy*, 2009, 39:290-298.
- Opalinska, J.B. et al. "Nucleic-Acid Therapeutics: Basic Principles and Recent Applications" *Nature Reviews: Drug Discovery*, Jul. 2002, 1:503-514.
- Park, D.S. et al. "Induction of ICAM-DR Molecules by IFN-Gamma and Oncogene Expression in Human Bladder Cancer Cell Lines" *Urologia Internationalis*, 1997, 59(2):72-80, abstract, 2 pages.
- Park, J.W. et al. "Primary Hepatocytes from Mice Treated with IL-2/IL-12 Produce T Cell Chemoattractant Activity that Is Dependent on Monokine Induced by IFN-gamma (Mig) and Chemokine Responsive to gamma-2 (Crg-2)" *The Journal of Immunology*, 2001, 166(6):3763-3770.
- Ralph, S.J. et al. "Resistance of Melanoma Cell Lines to Interferons Correlates with Reduction of IFN-Induced Tyrosine Phosphorylation" *The Journal of Immunology*, 1995, 154:2248-2256.
- Rebouillat, D. et al. "Characterization of the Gene Encoding the 100-kDa Form of Human 2',5' Oligoadenylate Synthetase" *Genomics*, 2000, 70:232-240.
- Reis, L.F.L. et al. "Critical role of a common transcription factor, IRF-1, in the regulation of IFN- β and IFN-inducible genes" *The EMBO Journal*, 1992, 11(1):185-193.
- Rosenblum, M.G. et al. "Differential Activity of the 30-kD and the 100-kD Forms of 2'-5' A_n Synthetase Induced by Recombinant Human interferon- α and Interferon- γ " *Journal of Interferon Research*, 1988, 8:275-282.
- Ruehlmann, J.M. et al. "MIG (CXCL9) Chemokine Gene Therapy Combines with Antibody-Cytokine Fusion Protein to Suppress Growth and Dissemination of Murine Colon Carcinoma" *Cancer Research*, Dec. 2001, 61:8498-8503.
- Sangfelt, O. et al. "Wild-Type p53-Induced Apoptosis in a Burkitt Lymphoma Cell Line is Inhibited by Interferon Gamma" *International Journal of Cancer*, 1996, 67:106-112.
- Sarkar, S.N. et al. "The Nature of the Catalytic Domain of 2'-5'-Oligoadenylate Synthetases" *The Journal of Biological Chemistry*, Sep. 1999, 274(35):25535-25542.
- Sarkar, S.N. et al. "Identification of the Substrate-binding Sites of 2'-5' Oligoadenylate Synthetase" *Journal of Biological Chemistry*, Jul. 5, 2002, 277(27):24321-24330.
- Sarkar, S.N. et al. "Crisscross Enzymatic Reaction between the Two Molecules in the Active Dimeric P69 Form of the 2'-5' Oligoadenylate Synthetase" *The Journal of Biological Chemistry*, Nov. 22, 2002, 277(47):44760-44754.
- Sarkar, S.N. et al. "Natural Mutations in a 2'-5' Oligoadenylate Synthetase Transgene Revealed Residues Essential for Enzyme Activity" *Biochemistry*, 2005, 44:6837-6843.
- Sarkar, S.N. et al. "Assays for the Interferon-Induced Enzyme 2',5' Oligoadenylate Synthetases" *Methods in Molecular Medicine*, 2005, 116:81-101.
- Saunders, N.A. et al. "Differential responsiveness of human bronchial epithelial cells, lung carcinoma cells, and bronchial fibroblasts to interferon-gamma in vitro" *Am J Respir Cell Mol Biol.*, Aug. 1994, 11(2):147-152.
- Schroder, H.C. et al. "Protection of HeLa-T4⁺ cells against human immunodeficiency virus (HIV) infection after stable transfection with HIV LTR-2',5'-oligoadenylate synthetase hybrid gene" *FASEB Journal*, Oct. 1990, 4:3124-3130.
- Seo, W.G. et al. "Synergistic cooperation between water-soluble chitosan oligomers and interferon-gamma for induction of nitric oxide synthesis and tumoricidal activity in murine peritoneal macrophages" *Cancer Letters*, 2000, 159:189-195.

(56)

References Cited**OTHER PUBLICATIONS**

- Sgadari, C. et al. "Interferon-inducible protein-10 identified as a mediator of tumor necrosis in vivo" *Proc. Natl. Acad. Sci. USA*, Nov. 1996, 93(24):13791-13796.
- Sgadari, C. et al. "MIG, the Monokine Induced By Interferon-gamma, Promotes Tumor Necrosis In Vivo" *Blood*, Apr. 15, 1997, 89(8):2635-2643.
- Sharma, S. et al. "SLC/CCL21-mediated anti-tumor responses require IFN γ , MIG/CXCL9 and IP-10/CXCL10" *Molecular Cancer*, 2003, 2:22, pp. 1-6.
- Shay, D.K. et al. "Bronchiolitis-Associated Mortality and Estimates of Respiratory Syncytial Virus-Associated Deaths among US Children, 1979-1997" *The Journal of Infectious Diseases*, 2001, 183:16-22.
- She, Y. et al. "The Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor ZD1839 Selectively Potentiates Radiation Response of Human Tumors in Nude Mice, with a Marked Improvement in Therapeutic Index" *Clinical Cancer Research*, Sep. 1, 2003, 9:3773-3778.
- Silverman, R.H. "Implications for Rnase L in Prostate Cancer Biology" *Biochemistry*, Feb. 25, 2003, 42(7):1805-1812.
- Smith, J.K. et al. "Oral Use of Interferon-alpha Stimulates ISG-15 Transcription and Production by Human Buccal Epithelial Cells" *Journal of Interferon and Cytokine Research*, 1999, 19:923-928.
- Strieter, R.M. et al. "Interferon Gamma-Inducible Protein 10 (IP-10), a Member of the C-X-C Chemokine Family, Is an Inhibitor of Angiogenesis" *Biochemical and Biophysical Research Communications* May 1995, 210(1):51-57.
- Sun, H. et al. "Interleukin-10 Gene Transfer Activates Interferon-gamma and the Interferon-gamma-Inducible Genes *Gbp-1/Mag-1* and *Mig-1* in Mammary Tumors" *International Journal of Cancer*, 1999, 80:624-629.
- Sun, H. et al. "Expression of the Chemokines IP-10 and Mig in IL-10 Transduced Tumors" *Journal of Immunology*, Mar. 2001, 24(2):138-143.
- Suzuki, M. et al. "Gene Therapy for Lung Diseases: Development in the Vector Biology and Novel Concepts for Gene Therapy Applications" *Current Molecular Medicine*, 2001, 1:67-79.
- Tajima, K. et al. "Interferon-gamma Differentially Regulates Susceptibility of Lung Cancer Cells to Telomerase-Specific Cytotoxic T Lymphocytes" *Int J. Cancer*, 2004, 110:403-412.
- Talpaz, M. et al. "Interferon-stimulated Genes in Interferon-sensitive and-resistant Chronic Myelogenous Leukemia Patients" *Cancer Research*, Mar. 1992, 52:1087-1090.
- Tannenbaum, C.S. et al. "The CXC Chemokines IP-10 and Mig Are Necessary for IL-12-Mediated Regression of the Mouse RENCA Tumor" *The Journal of Immunology*, 1998, 161:927-932.
- Villagomez, M.T. et al. "Tumour necrosis factor-alpha but not interferon-gamma is the main inducer of inducible protein-10 in skin fibroblasts from patients with atopic dermatitis" *British Journal of Dermatology*, 2004, 150:910-916.
- Witt, P.L. et al. "Isoforms p69 and p100 of 2',5'-Oligoadenylate Synthetase Induced Differentially by Interferons In Vivo and In Vitro" *Journal of Interferon Research*, 1993, 13:17-23.
- Witzenrath, M. et al. "Detection of allergen-induced airway hyper-responsiveness in isolated mouse lungs" *Am J Physiol Lung Cell Mol Physiol*, 2006, 291:L466-L472.
- Woolhiser, M.R. et al. "Immunological Responses of Mice following Administration of Natural Rubber Latex Proteins by Different Routes of Exposure" *Toxicological Sciences*, 2000, 55:343-351.
- Yu, F. et al. "Protein Kinase C Is Required for Induction of 2',5'-Oligoadenylate Synthetases" *Experimental Cell Research*, 1997, 234:240-248.
- Yu-Lee, L.Y. et al. "Interferon-Regulatory Factor 1 Is an Immediate-Early Gene under Transcriptional Regulation by Prolactin in Nb2 T Cells" *Molecular and Cellular Biology*, Jun. 1990, 10(6):3087-3094.
- An, Z. et al. "Interferon Gamma is Highly Effective Against Orthotopically-Implanted Human Pleural Adenocarcinoma in Nude Mice" *Anticancer Research*, 1996, 16:2546-2552.
- Iqbal, M. et al. "Nasal delivery of chitosan-DNA plasmid expressing epitopes of respiratory syncytial virus (RSV) induces protective CTL responses in BALB/c mice" *Vaccine*, 2003, 21:1478-1485.
- Nanni, P. et al. "Therapy of murine mammary carcinoma metastasis with interferon γ and MHC gene-transduced tumour cells" *British Journal of Cancer*, 1996, 74:1564-1569.
- Smyth, M. et al. "Tumor Necrosis Factor-related Apoptosis-inducing Ligand (TRAIL) contributes to Interferon γ -dependent Natural Killer Cell Protection from Tumor Metastasis" *J. Exp. Med.*, 2001, 193:661-670.
- Office Action mailed Sep. 26, 2011 in U.S. Appl. No. 11/117,169, filed Apr. 27, 2005.

* cited by examiner

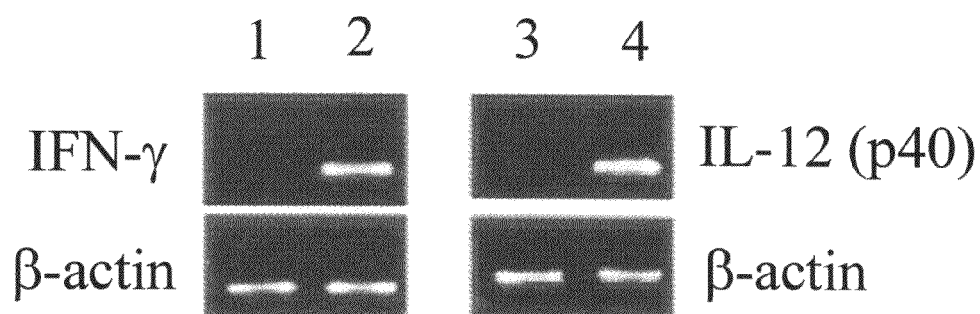
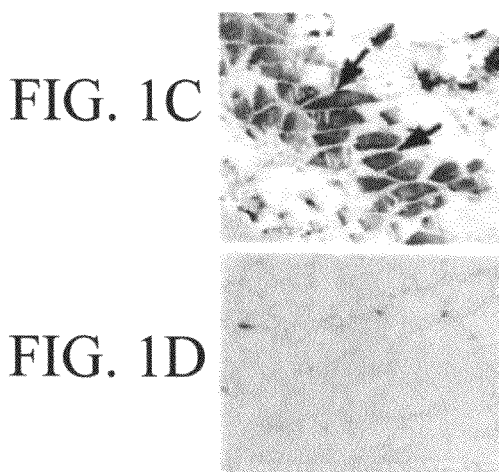
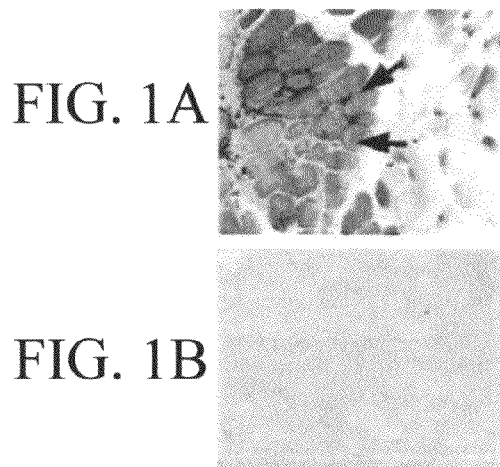


FIG. 1E

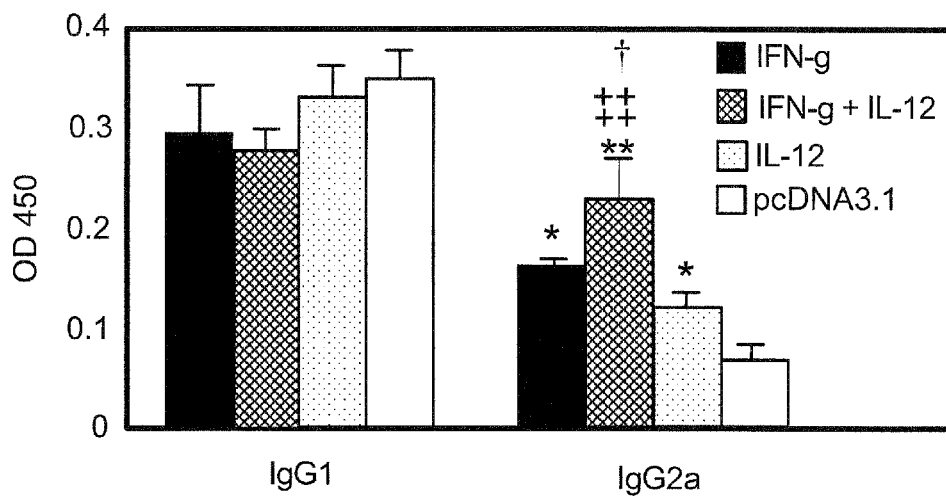


FIG. 2A

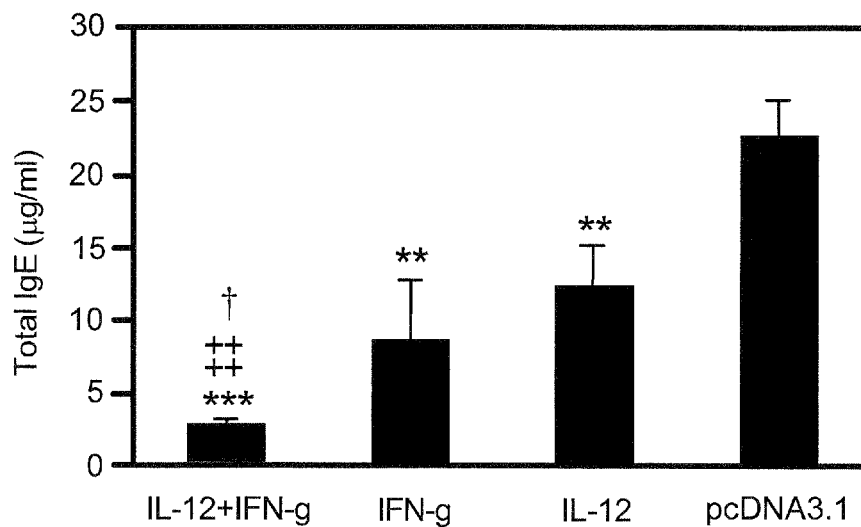


FIG. 2B

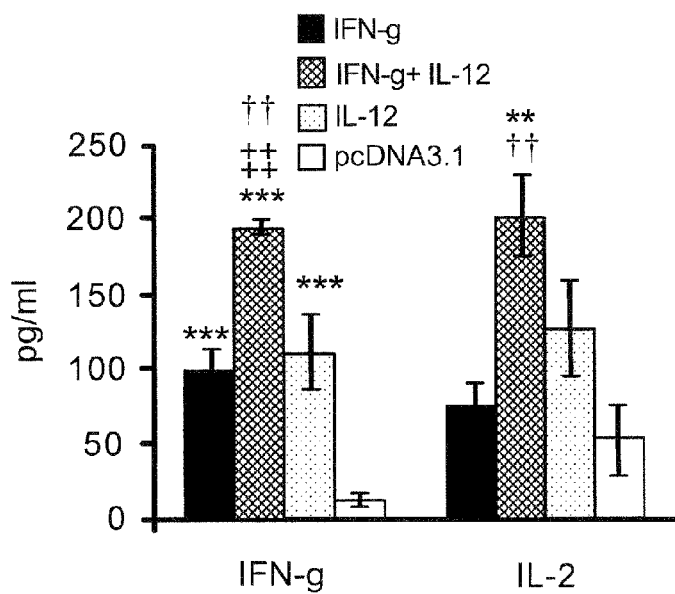


FIG. 3A

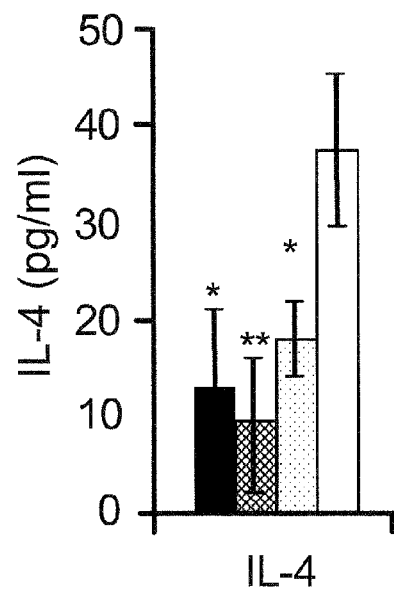


FIG. 3B

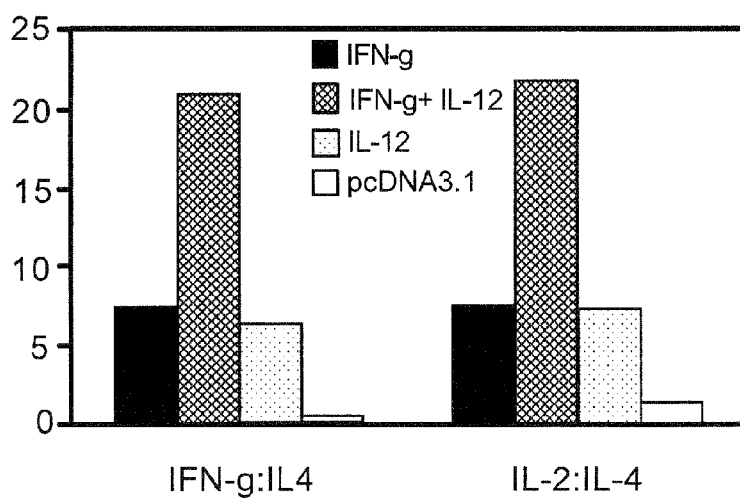


FIG. 3C

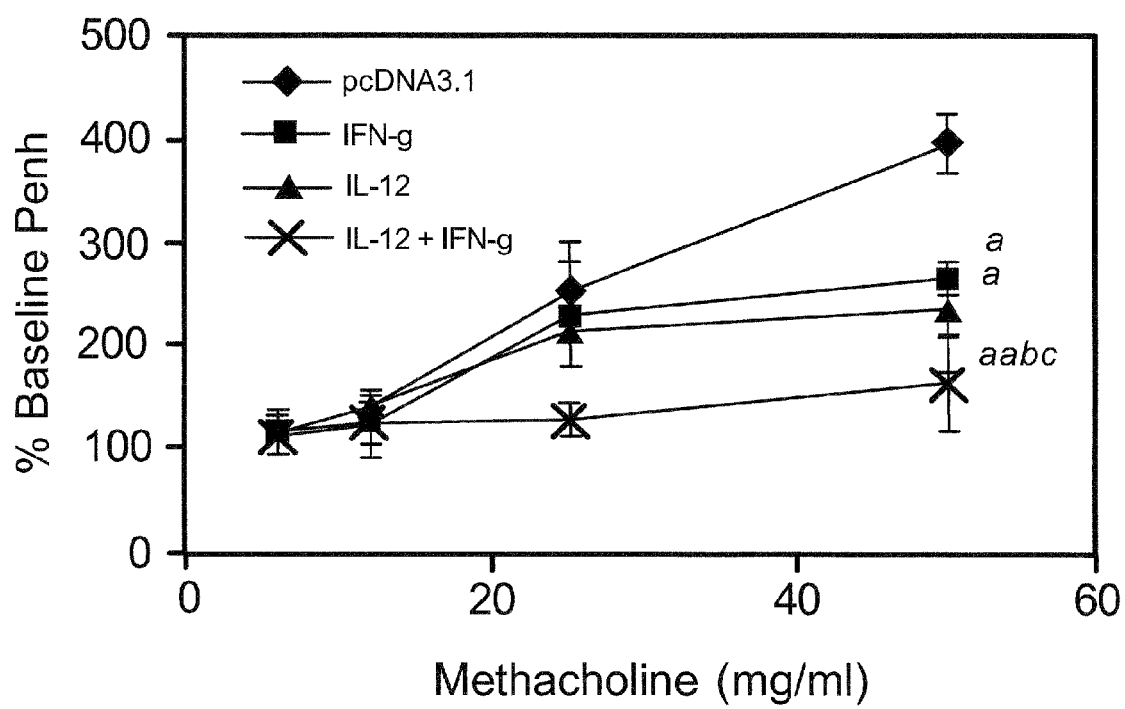


FIG. 4

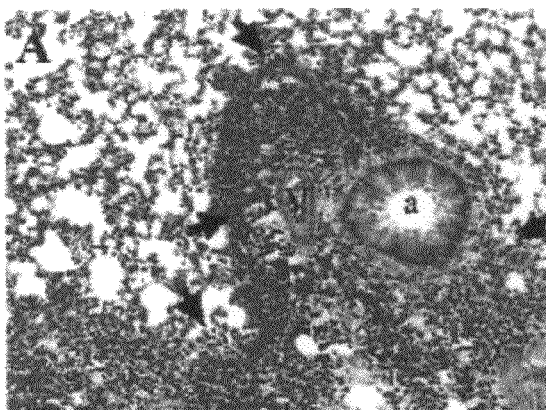


FIG. 5A

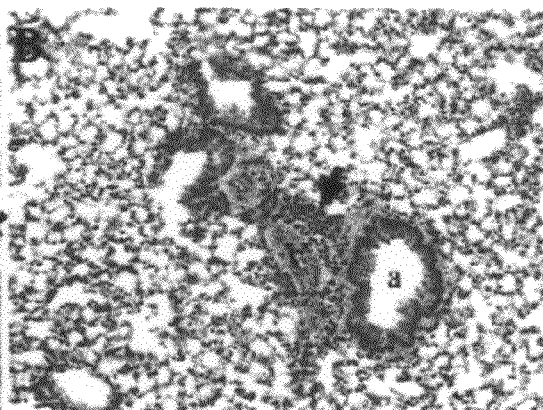


FIG. 5B

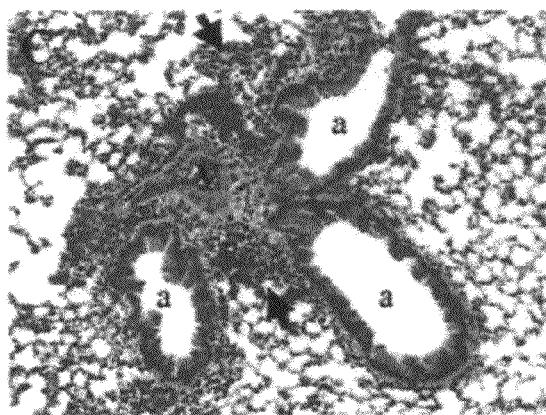


FIG. 5C

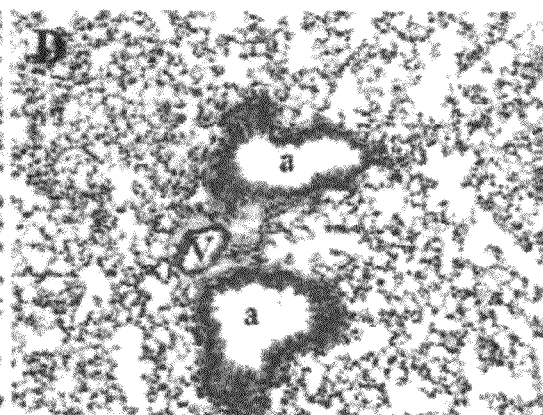


FIG. 5D

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GENETIC ADJUVANTS FOR IMMUNOTHERAPY

CROSS-REFERENCE TO RELATED APPLICATION

The present application is a continuation of U.S. patent application Ser. No. 10/655,873, filed Sep. 5, 2003, now U.S. Pat. No. 7,595,303, which claims the benefit of U.S. Provisional Application Ser. No. 60/319,523, filed Sep. 5, 2002. Each of these applications is incorporated herein by reference in its entirety, including any figures, tables, nucleic acid sequences, amino acid sequences, or drawings.

BACKGROUND OF INVENTION

The effectiveness of allergen immunotherapy (AIT) is often compromised by the use of high doses of allergens for treatment, which can lead to severe systemic reactions and the inconvenience and discomfort of frequent dosing (Kemp, S. F., *Immunol Allergy Clin North Am*, 2000, 20:571). It has been suggested that the utilization of an adjuvant in conjunction with an allergen vaccine might enhance both the safety and the effectiveness of AIT (Mohapatra, S. S. and San Juan, H. *Immunol Allergy Clin North Am*, 2000, 20:625-642). Two approaches are under investigation. The first is coimmunization with a mixture of antigen(s) and bacterial DNA or immunostimulatory oligodeoxynucleotides (ODNs), which contain CpG motifs (Horner, A. A. et al., *J Allergy Clin Immunol*, 2000, 106:349-356) and elicit a protective T_H1 response (Roman, M. et al., *Nat Med*, 1997, 3:849-854). The potential of CpG-containing ODNs as adjuvants in AIT is under intense investigation; however, it has been reported that the immunostimulatory activity of these ODNs might be blocked by certain non-CpG motifs (Krieg, A. M. et al., *Proc Natl Acad Sci USA*, 1998, 95:12631-12636; Hacker, H. et al., *EMBO J*, 1998, 17:6230-6240). In addition, these ODNs might not suppress established allergic responses (Peng, Z. et al., *Int Immunol*, 2001, 13:3-11). In a second approach, the potential of plasmid DNA (pDNA)-encoding cytokine(s) as genetic adjuvants has been examined, with varying levels of success, for modulating the immune response stimulated by administered antigen vaccines (Pasquini, S. et al, *Immunol Cell Biol*, 1997, 75:397-401).

Cytokines interferon- γ (IFN- γ) and interleukin-12 (IL-12) are known to mediate T-cell differentiation toward a T_H1 -like phenotype (Boehm, U. et al., *Annu Rev Immunol*, 1997, 15:749-795). Despite numerous studies in which pure or recombinant IFN- γ and IL-12 were used, their in vivo use has been limited by the short half-life of these molecules and the associated severe adverse effects (Mohapatra, S. S., *Science*, 1995, 269:1499). Mucosal IFN- γ gene transfer has earlier been shown to inhibit both antigen- and T_H2 cell-induced pulmonary eosinophilia and airway hyper-reactivity (Li, X. M. et al., *J. Immunol.*, 1996, 157:3116-3219). Vaccinia virus-mediated IL-12 gene transfer to the mouse airway abrogated airway eosinophilia and IgE synthesis (Hogan, S. P. et al, *Eur J. J. Immunol.*, 1998, 28:413-423). However, the direct effects of these cytokine plasmids as genetic adjuvants in the allergen vaccines used for AIT have not been addressed.

In a previous study, a combination of allergen and IFN- γ effectively redirected allergen-specific T-cell cytokine production toward elevated IFN- γ production in human PBMC cultures (Paironchi, P. et al, *Eur J Immunol*, 1996, 26:697-703). In a murine model of Kentucky blue grass (KBG) allergy, parenteral administration of 1 mg of recombinant allergen induced effective immune deviation (Cao, Y. et al.,

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Immunology, 1997, 90:46-51). Cytokine gene transfer studies have been carried out (Li, X. M. et al., *J. Immunol.*, 1996, 157:3116-3219; Hogan, S. P. et al., *Eur. J. Immunol.*, 1998, 28:413-423).

It would be advantageous to have the capability to mediate T-cell differentiation toward a T_H1 -like phenotype using IFN- γ and/or IL-12, thereby enhancing the efficacy of allergen vaccines, without limitation by the short half-life of these molecules and the associated severe adverse effects.

BRIEF SUMMARY

The present invention pertains to methods and pharmaceutical compositions for modulating an immune response. The method of the present invention involves administration of an effective amount of nucleic acid molecules encoding interleukin-12 (IL-12), interferon-gamma (IFN- γ), or a combination thereof, to a patient in need of such treatment. In one embodiment, the nucleic acid molecules encoding IL-12 and/or IFN- γ are co-administered with an antigen.

The present invention further concerns pharmaceutical compositions comprising a nucleic acid sequence encoding IL-12 and/or IFN- γ and a pharmaceutically acceptable carrier. Preferably, the composition contains a nucleic acid sequence encoding both IL-12 and IFN- γ , and a pharmaceutically acceptable carrier. Optionally, in addition to a nucleic acid sequence encoding IL-12 and/or IFN- γ , the pharmaceutical composition of the present invention contains an antigen.

In another aspect, the present invention concerns expression vectors containing a nucleotide sequence encoding IL-12 and IFN- γ , and an operably-linked promoter sequence. In another aspect, the present invention concerns cells genetically modified with a nucleotide sequence encoding IL-12 and IFN- γ .

BRIEF DESCRIPTION OF DRAWINGS

The file of this patent contains at least one drawing executed in color. Copies of this patent with color drawings will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

FIGS. 1A-1E show expression of murine IFN- γ and IL-12 p40 subunit in the mouse muscle. Mice were vaccinated as described in the Methods section. Seven days after the last DNA injection, expression for IFN- γ and IL-12 p40 subunit was checked by immunohistochemistry (FIGS. 1A-1D) and RT-PCR (FIG. 1E). Mice vaccinated with pIFN- γ (FIG. 1A) and pIL-12 (FIG. 1C) showed expression of IFN- γ and IL-12 p40 subunits, respectively, as indicated by positive staining (arrows). Mice vaccinated with control empty vector did not show expression for IFN- γ (FIG. 1B) and IL-12 p40 (FIG. 1D). In FIG. 1E, total RNA was isolated from the muscle, and RT-PCR was performed for IFN- γ and IL-12 p40 subunit. Mice vaccinated with pIFN- γ and pIL-12 showed IFN- γ (panel 1, lane 2) and IL-12 p40-specific mRNA amplification (panel 1, lane 4). No amplification was observed in the mice vaccinated with control empty vector (panel 1, lanes 1 and 3). β -actin was used as internal control (lower panel).

FIGS. 2A-2B show analysis of the total IgE and KBG-specific IgG subtypes. Four groups of mice (n=6) were vaccinated as described in the Methods section. On day 21, after immunization with alum and KBG allergen, their serum was analyzed for total IgE (FIG. 2B) and KBG-specific IgG2a and IgG1 (FIG. 2A) antibodies by ELISA. Bars represent the means \pm SDs. *P<0.05; **P<0.01; ***P<0.001 in comparison

with pcDNA3.1 group. ^{*}P<0.05; ^{**}P<0.01; ^{***}P<0.001 in comparison with pIFN- γ . ^{*}P<0.05; ^{**}P<0.01; ^{***}P<0.001 in comparison with pIL-12 group.

FIGS. 3A-3C show analysis of cytokine production and dominant cytokine pattern following cytokine-encoding DNA vaccination. FIGS. 3A and 3B show analysis of cytokine production. Mice (n=6) were vaccinated as described in the Methods section. On day 7 after KBG and alum immunization, their spleens were cultured in vitro for 48 hours in the presence of KBG allergen and cytokines were measured by ELISA. Bars represent the means \pm SDs. ^{*}P<0.05; ^{**}P<0.01; ^{***}P<0.001 in comparison with pcDNA3.1 group. ^{*}P<0.05; ^{**}P<0.01; ^{***}P<0.001 in comparison with pIFN- γ (IFN-g) group. ^{*}P<0.05; ^{**}P<0.01; ^{***}P<0.001 in comparison with pIL-12 (IL-12) group. FIG. 3C shows analysis of the dominant cytokine pattern after cytokine DNA vaccination. Dominant cytokine pattern was determined from the IFN- γ /IL-4 and IL-2/IL-4 ratios. Bars represent means.

FIG. 4 shows measurement of the airway hyperresponsiveness in KBG-sensitized and -challenged mice after cytokine DNA vaccination. Naive mice (n=4) were vaccinated as described in the Methods section and sensitized with the allergen 7 days later. Ten days after the sensitization, animals were challenged intranasally 3 times with 50 μ g of KBG allergen. Airway reactivity to inhaled methacholine (6 to 50 mg/mL) was measured 24 hours later. Results are expressed as means \pm SDs of enhanced pause values. a, P<0.05; aa, P<0.01 in comparison with pcDNA3.1 group. b and c, P<0.05 in comparison with pIFN- γ (IFN-g) and pIL-12 (IL-12) groups, respectively.

FIGS. 5A-5D show assessment of the lung inflammation in KBG-sensitized and -challenged mice after cytokine DNA vaccination. Lung tissue was removed from the different groups of mice (n=4) 24 hours after the last intranasal allergen challenge and was stained with hematoxylin and eosin. A representative photomicrograph from each group is shown (FIG. 5A: pcDNA3.1; FIG. 5B: pIFN- γ ; FIG. 5C: pIL-12; FIG. 5D: pIFN- γ +pIL-12). Arrows indicated cellular infiltration. a, airway; v, vessel.

BRIEF DESCRIPTION OF SEQUENCES

SEQ ID NO:1 is a forward primer for the murine IL-12 p40 subunit.

SEQ ID NO:2 is a reverse primer for murine IL-12 p40 subunit.

SEQ ID NO:3 is a forward primer to generate the plasmid pc40.

SEQ ID NO:4 is a reverse primer to generate the plasmid pc40.

SEQ ID NO:5 is a forward primer for murine IL-12 p35 subunit.

SEQ ID NO:6 is a reverse primer for murine IL-12 p35 subunit.

SEQ ID NO:7 is the nucleotide sequence encoding the human IL-12 p35 subunit.

SEQ ID NO:8 is the amino acid sequence of the human IL-12 p35 subunit.

SEQ ID NO:9 is the nucleotide sequence of the human IL-12 p40 subunit.

SEQ ID NO:10 is the amino acid sequence of the human IL-12 p40 subunit.

SEQ ID NO: 11 is the nucleotide sequence encoding human IFN- γ .

SEQ ID NO:12 is the amino acid sequence of human IFN- γ .

DETAILED DISCLOSURE

The present invention pertains to adjuvantation using nucleic acid molecules encoding IL-12, IFN- γ , or both IL-12 and IFN- γ . In one embodiment, the nucleic acid molecules encoding IL-12 and/or IFN- γ are co-administered with an antigen of the pathogen in order to modulate an immune response to a pathogen (one or more) in a patient (human or non-human mammal). Preferably, effective amounts of nucleic acid sequences encoding both IL-12 and IFN- γ are administered simultaneously or sequentially to a patient in need of such treatment. More preferably, effective amounts of nucleic acid sequences encoding both IL-12 and IFN- γ are administered simultaneously or sequentially to a patient, and an antigen is administered simultaneously or sequentially to the patient.

As described herein, the "immune response" modulated in the patient can be, for example, a cytokine immune response and/or a humoral immune response (e.g., antigen-specific). As used herein, the term "modulate", and grammatical variations thereof, is intended to mean strengthening (i.e., augmenting) or weakening (i.e., lessening, inhibiting) of an immune response within a patient. In one embodiment, the immune response of the patient is modulated such that production of IgE antibodies is inhibited. In further embodiments, the cytokine profiles of the patient's cells are altered to produce more T_H1-like cytokines (e.g., interleukin-2 (IL-2) and IFN- γ) and less T_H2-like cytokines ((interleukin-4) IL-4). The altered cytokine profile is more apparent in patients administered nucleic acid sequences encoding both IL-12 and IFN- γ . Depending upon the particular antigen that the patient is administered or otherwise exposed to (e.g., an allergen), the nucleic acid sequences encoding IL-12 and/or IFN- γ may inhibit or prevent airway hyper-responsiveness and inflammation.

Administration of nucleic acid molecules encoding IL-12 and/or IFN- γ , or biologically active fragments or homologs thereof, according to the subject invention, may be used to modulate the immune response in order to prevent or treat immune-related or inflammatory-related conditions such as, but not limited to, allergies, allergic rhinitis, atopic dermatitis, asthma, allergic sinusitis, pulmonary fibrosis, and cancer.

In other embodiments, the nucleic acid molecules used in the methods, vectors, cells, and compositions of the present invention encode IL-12-like molecules and/or IFN- γ -like molecules. As used herein, the terms "IL-12-like molecule" and "IFN- γ -like molecule" refers to polypeptides exhibiting IL-12-like activity and IFN- γ -like activity, respectively, when the nucleic acid molecule encoding the polypeptide is expressed, as can be determined in vitro or in vivo. For purposes of the subject invention, IL-12-like activity and IFN- γ -like activity refer to those polypeptides having one or more of the functions of the native IL-12 or IFN- γ cytokine, such as the capability to alter the cytokine profile of a patient's cells to produce more T_H1-like cytokines (e.g., interleukin-2 (IL-2) and IFN- γ) and less T_H2-like cytokines ((interleukin-4) IL-4)). An example of an IL-12-like molecule is interleukin-23 (IL-23). Examples of IFN- γ -like molecules are interferon-alpha (IFN- α) and interferon-beta (IFN- β), including biologically active fragments thereof.

Following administration, the efficacy of the cytokine-encoding nucleic acid sequences can be assessed by specific cytokine production or antibody production, for example. One of ordinary skill in the art can assess these parameters using conventional methods.

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A used herein, the term "antigen" is intended to mean one or more immunostimulatory agents capable of being bound by a selective binding agent, such as an antibody, and additionally capable of being used in an animal to produce antibodies capable of binding to an epitope of that antigen. An antigen may have one or more epitopes. Antigens can be molecules or portions of molecules including, but not limited to, proteins or fragments thereof (e.g., proteolytic fragments), peptides (e.g., synthetic peptides, polypeptides), glycoproteins, carbohydrates (e.g., polysaccharides), lipids, glycolipids, hapten conjugates, recombinant nucleotides (e.g., recombinant DNA), whole organisms (killed or attenuated) or portions thereof, toxins and toxoids (e.g., tetanus, diphtheria, cholera) and/or organic molecules. Particular examples of antigens for use in the present invention include allergens, such as Kentucky blue grass (KBG) allergen.

The antigen can be obtained or derived from a variety of pathogens or organisms, such as bacteria (e.g., *Bacillus*, Group B *Streptococcus*, *Bordetella*, *Listeria*, *Bacillus anthracis*, *S. pneumoniae*, *N. meningitidis*, *H. influenza*), viruses (e.g., hepatitis, measles, poliovirus, human immunodeficiency virus, influenza virus, parainfluenza virus, respiratory syncytial virus), mycobacteria (*M. tuberculosis*), parasites (*Leishmania*, *Schistosomes*, *Tranpanosomes*, *Toxoplasma*, *pneumocystis*) and fungi (e.g., *Candida*, *Cryptococcus*, *Coccidioides*, *Aspergillus*), against which an immune response is desired in a patient. The antigen of a pathogen can be obtained using skills known in the art. For example, the antigen can be isolated (purified, essentially pure) directly from the pathogen, derived using chemical synthesis, or obtained using recombinant methodology. In addition, the antigen can be obtained from commercial sources. A suitable antigen for use in the present invention can include at least one B and/or T cell epitope (e.g., T helper cell or cytolytic T cell epitope). Other suitable antigens useful in the compositions of the present invention can be determined by those of skill in the art.

The IL-12-encoding nucleic acid sequence and IFN- γ -encoding nucleic acid sequence can be within the same nucleic acid molecule or separate nucleic acid molecules. Furthermore, it will be understood that a nucleic acid sequence can be a multimer comprising repeating units of the IL-12-encoding nucleic acid sequence and/or IFN- γ -encoding nucleic acid sequence (i.e., homopolymers or heteropolymers) for enhanced expression of the IL-12 and/or IFN- γ coding sequences, or biologically active fragments or homologs thereof.

As used herein, the term "co-administer", and grammatical variations thereof, is intended to mean administration of agents (e.g., nucleic acids, antigens, etc.) simultaneously or sequentially.

The nucleic acid sequences encoding IL-12 and/or IFN- γ used in the methods, expression vectors, and pharmaceutical compositions of the present invention are preferably isolated. According to the present invention, an isolated nucleic acid molecule or nucleic acid sequence is a nucleic acid molecule or sequence that has been removed from its natural milieu. As such, "isolated" does not necessarily reflect the extent to which the nucleic acid molecule has been purified. An isolated nucleic acid molecule or sequence useful in the present composition can include DNA, RNA, or any derivatives of either DNA or RNA. An isolated nucleic acid molecule or sequence can be double stranded (i.e., containing both a coding strand and a complementary strand) or single stranded.

Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases are

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used interchangeably herein. As used herein, a "coding" nucleic acid sequence refers to a nucleic acid sequence that encodes at least a portion of a peptide or protein (e.g., a portion of an open reading frame), and can more particularly refer to a nucleic acid sequence encoding a peptide or protein which, when operably-linked to a transcription control sequence (e.g., a promoter sequence), can express the peptide or protein. A translation initiation codon can be inserted as necessary, making methionine the first amino acid in the sequence. Optionally, the IL-12 and/or IFN- γ encoding nucleic acid sequences used in the subject invention include a sequence encoding a signal peptide upstream of the cytokine(s)-encoding sequence(s), thereby permitting secretion of the IL-12 and/or IFN- γ , or a biologically active fragment thereof, from a host cell.

The term "operably-linked" is used herein to refer to an arrangement of flanking sequences wherein the flanking sequences so described are configured or assembled so as to perform their usual function. Thus, a flanking sequence operably-linked to a coding sequence may be capable of effecting the replication, transcription and/or translation of the coding sequence. For example, a coding sequence is operably-linked to a promoter when the promoter is capable of directing transcription of that coding sequence. A flanking sequence need not be contiguous with the coding sequence, so long as it functions correctly. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence, and the promoter sequence can still be considered "operably-linked" to the coding sequence. Each nucleotide sequence coding for IL-12 or IFN- γ will typically have its own operably-linked promoter sequence.

The term "vector" is used to refer to any molecule (e.g., nucleic acid, plasmid, or virus) used to transfer coding information (e.g., nucleic acid sequence encoding IL-12 and/or IFN- γ) to a host cell. The term "expression vector" refers to a vector that is suitable for use in a host cell (e.g., patient's cell) and contains nucleic acid sequences which direct and/or control the expression of heterologous nucleic acid sequences. Expression includes, but is not limited to, processes such as transcription, translation, and RNA splicing, if introns are present. A great variety of expression vectors can be used to produce IL-12 and/or IFN- γ , or biologically active fragments thereof. IL-12 and/or IFN- γ -encoding nucleic acid sequences can be modified according to methods known in the art to provide optimal codon usage for expression in a particular expression system.

In another aspect, the present invention includes pharmaceutical compositions comprising a nucleic acid sequence encoding IL-12 and/or IFN- γ and a pharmaceutically acceptable carrier. Preferably, the composition contains a nucleic acid sequence encoding both IL-12 and IFN- γ , and a pharmaceutically acceptable carrier. The nucleic acid sequence encoding IL-12 and/or IFN- γ may be contained within an expression vector, such as a DNA plasmid or viral vector (e.g., retrovirus, modified herpes virus, herpes virus, adenovirus, adeno-associated virus, and the like). Where a combination of nucleic acid sequences encoding IL-12 and IFN- γ are to be administered to a patient, sequences may be contained within one vector (e.g., a DNA plasmid) or separate vectors (e.g., separate plasmids), or types of vectors. Optionally, the pharmaceutical composition of the present invention further includes an antigen.

The pharmaceutical compositions of the subject invention can be formulated according to known methods for preparing pharmaceutically useful compositions. Furthermore, as used herein, the phrase "pharmaceutically acceptable carrier"

means any of the standard pharmaceutically acceptable carriers. The pharmaceutically acceptable carrier can include diluents, adjuvants, and vehicles, as well as implant carriers, and inert, non-toxic solid or liquid fillers, diluents, or encapsulating material that does not react with the active ingredients of the invention. Examples include, but are not limited to, phosphate buffered saline, physiological saline, water, and emulsions, such as oil/water emulsions. The carrier can be a solvent or dispersing medium containing, for example, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. Formulations are described in a number of sources that are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Sciences* (Martin E W [1995] Easton Pa., Mack Publishing Company, 19th ed.) describes formulations which can be used in connection with the subject invention. Formulations suitable for parenteral administration include, for example, aqueous sterile injection solutions, which may contain antioxidants, buffers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the condition of the sterile liquid carrier, for example, water for injections, prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powder, granules, tablets, etc. It should be understood that in addition to the ingredients particularly mentioned above, the formulations of the subject invention can include other agents conventional in the art having regard to the type of formulation in question. The pharmaceutical composition can be adapted for various forms of administration. Administration can be continuous or at distinct intervals as can be determined by a person skilled in the art.

The administration of the IL-12 and/or IFN- γ -encoding nucleic acid sequences are administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual patient, the site and method of administration, scheduling of administration, patient age, sex, body weight, and other factors known to medical practitioners. The pharmaceutically "effective amount" for purposes herein is thus determined by such considerations as are known in the art. A therapeutically effective amount of IL-12 and/or IFN- γ -encoding nucleic acid molecules is that amount necessary to provide a therapeutically effective amount of the corresponding polypeptide(s), when expressed in vivo. The amount of IL-12 and/or IFN- γ must be effective to achieve a modulated immune response, including but not limited to total prevention of (e.g., protection against) pathogen infection and/or allergic response, and to improved survival rate or more rapid recovery, or improvement or elimination of symptoms associated with pathogen infection or allergic response, and other indicators as are selected as appropriate measures by those skilled in the art. In accordance with the present invention, a suitable single dose size is a dose that is capable of preventing or alleviating (reducing or eliminating) a symptom in a patient when administered one or more times over a suitable time period. One of skill in the art can readily determine appropriate single dose sizes for systemic administration based on the size of a mammal and the route of administration.

Mammalian species which benefit from the disclosed compositions and methods include, and are not limited to, apes, chimpanzees, orangutans, humans, monkeys; domesticated

animals (e.g., pets) such as dogs, cats, guinea pigs, hamsters, Vietnamese pot-bellied pigs, rabbits, and ferrets; domesticated farm animals such as cows, buffalo, bison, horses, donkey, swine, sheep, and goats; exotic animals typically found in zoos, such as bear, lions, tigers, panthers, elephants, hippopotamus, rhinoceros, giraffes, antelopes, sloth, gazelles, zebras, wildebeests, prairie dogs, koala bears, kangaroo, opossums, raccoons, pandas, hyena, seals, sea lions, elephant seals, otters, porpoises, dolphins, and whales. As used herein, the term "patient" is intended to include such human and non-human mammalian species. Nucleic acid molecules encoding IL-12 and/or IFN- γ , or biologically active fragments thereof, can be administered to patients of the same species or different species from which the nucleic acid molecules naturally exist, for example.

The nucleic acid sequences encoding IL-12 and/or IFN- γ (and pharmaceutical compositions containing them) can be administered to a patient by any route that results in modulated immune response. For example, the genetic material can be administered intravenously (I.V.), intramuscularly (I.M.), subcutaneously (S.C.), intradermally (I.D.), orally, intranasally, etc.

Examples of intranasal administration can be by means of a spray, drops, powder or gel and also described in U.S. Pat. No. 6,489,306, which is incorporated herein by reference in its entirety. One embodiment of the present invention is the administration of the invention as a nasal spray. Alternate embodiments include administration through any oral or mucosal routes, sublingual administration, and even eye drops. However, other means of drug administrations are well within the scope of the present invention.

The present invention also encompasses combination therapy. By combination therapy is meant that nucleic acid sequences encoding IL-12 and/or IFN- γ can be administered in combination with other biologically active agents, such as antigens, other immunomodulators or immunostimulatory molecules, such as interferons or interleukens, and antimicrobial agents, such as antibiotics, antifungal drugs, antiviral drugs, etc.

The present invention can be conjugated with chitosan or chitosan derivatives. For example, DNA chitosan nanoparticles can be generated, as described by Roy, K. et al. (*Nat Med*, 1999, 5:387). Chitosan allows increased bioavailability of the nucleic acid sequences because of protection from degradation by serum nucleases in the matrix and thus has great potential as a mucosal gene delivery system. Chitosan also has many beneficial effects, including anticoagulant activity, wound-healing properties, and immunostimulatory activity, and is capable of modulating immunity of the mucosa and bronchus-associated lymphoid tissue. In one embodiment of the present invention, chitosan derived nanoparticles are used as adjuvants or conjugates.

IL-12 is a heterodimeric cytokine that has a molecular weight of 75 kDa and is composed of disulfide-bonded 40 kDa and 35 kDa subunits. As used herein, "interleukin-12" and "IL-12" refer to interleukin 12 protein, its individual subunits, multimers of its individual subunits, biologically active fragments of IL-12, and biologically active homologs of "interleukin-12" and "IL-12", such as mammalian homologs. As defined herein, biologically active fragments of IL-12 are fragments that, for example, modulate an immune response to an antigen in a patient who has been administered or otherwise exposed to the antigen. As also defined herein, biologically active fragments or homologs of "interleukin-12" and "IL-12" include modified IL-12 protein such that the resulting IL-12 product has immune response modulation activity similar to the IL-12 described herein (e.g., the ability

to modulate an immune response to an antigen, when administered with the antigen, in a patient, relative to in vivo conditions in the absence of the cytokine or relative to administration of the antigen alone). Biologically active homologs or fragments of “interleukin-12” also include nucleic acid sequences (e.g., DNA, RNA) and portions thereof, which encode a protein or peptide having the IL-12 function or activity described herein (e.g., the ability to modulate an immune response to an antigen, when administered with the antigen, in a patient). In addition, the term includes a nucleotide sequence which, through the degeneracy of the genetic code, encodes a similar peptide gene product as IL-12 and has

the IL-12 activity described herein. For example, a homolog of “interleukin-12” and “IL-12” includes a nucleotide sequence which contains a “silent” codon substitution (e.g., substitution of one codon encoding an amino acid for another codon encoding the same amino acid) or an amino acid sequence which contains a “silent” amino acid substitution (e.g., substitution of one acidic amino acid for another acidic amino acid).

An exemplified nucleotide sequence encodes the human IL-12 p35 subunit (Accession No: NM_000882, NCBI database, which is hereby incorporated by reference in its entirety):

(SEQ ID NO: 7)

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1  ttctattttg ggccgagctg gagcgggcgg gcccgcccc gaacggctgc ggccgggcac
61  cccgggagtt aatccgaaag cgccgcaagc cccgcgggcc ggccgcaccg cagctgtcac
121 cgagaagctg atgtagagag agacacagaa ggagacagaa agcaagagac cagagtcccc
181 ggaaagtctc gccgcgcctc gggacaatta taaaaatgtg gcccccctggg tcagcctccc
241 agccaccgcc ctcacctgcc gcggccacag gtctgcatcc agcggtctgc cctgtgtccc
301 tgcagtgcgg gctcagcatg tgtccagcgc gcagcctcct ccttgtggct acctgtgtcc
361 tcctggacca cctcagtttg gccagaaaac tccccgtggc cactccagac ccaggaatgt
421 tcccatgcct tcaccactcc caaaacctgc tgaggggcgt cagcaacatg ctccagaagg
481 ccagacaaa cctagaatct tacccttgca cttctgaaga gattgatcat gaagatatca
541 caaaagataa aaccagcaca gtggaggcct gtttaccatt ggaattaacc agaagtgaga
601 gttgcctaaa ttccagagag acctctttca taactaatgg gagttgcctg gcctccagaa
661 agacctcttt tatgatggcc ctgtgcctta gtagtattta tgaagacttg aagatgtacc
721 aggtggagtt caagaccatg aatgcaaagc ttctgatgga tcctaagagg cagatctttc
781 tagatcaaaa catgctggca gttattgatg agctgatgca ggccctgaat ttcaacagtg
841 agactgtgcc acaaaaatcc tcccttgaa gaaacggattt ttataaaact aaaatcaagg
901 tctgcatact tcttcatgct ttcagaattc gggcagtgac tattgataga gtgatgagct
961 atctgaatgc ttcctaaaaa gcgaggctcc tccaaaccgt tgtcattttt ataaaacttt
1021 gaaatgagga aactttgata ggatgtggat taagaactag ggagggggaa agaaggatgg
1081 gactattaca tccacatgat acctctgata aagtattttt gacattttact gtggataaat
1141 tgtttttaag ttttcatgaa tgaattgcta agaagggaaa atatccatcc tgaagggtgt
1201 ttctattcac tttaatagaa gggcaaatat ttataagcta tttctgtacc aaagtgtttg
1261 tggaacaaa catgtaagca taacttattt taaaatattt atttatataa cttggtaatc
1321 atgaaagcat ctgagctaac ttatatttat ttatgttata ttattataat tatttatcaa
1381 gtgtatttga aaaatatttt taagtgttct aaaaataaaa gtattgaatt aaagtgaaaa
1441 aaaa

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(SEQ ID NO: 8)

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MWPPGSASQPPSPAAATGLHPARPVSLQCRLSMCPARSLLLVATLVLLDHLSLARNLPVATPDGPMFP
CLHHSQNLLRAVSNMLQKARQTFEYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFIT
NGSCLASRKTSFMMALCLSIYEDLMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSET
VPQKSSLEEDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

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A further exemplified nucleotide sequence encodes the human IL-12 p40 subunit (Accession No: NM_002187, NCBI database, which is hereby incorporated by reference in its entirety):

(SEQ ID NO: 9)

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1  ctgtttcagg gccattggac tctccgtcct gccagagca agatgtgtca ccagcagttg
61  gtcattctctt ggttttccct gggtttttctg gcattctccc tcgtggccat atgggaactg
121 aagaaagatg tttatgtcgt agaattggat tggatccgg atgccctgg agaaatggtg
181 gtctctacct gtgacacccc tgaagaagat ggtatcacct ggaccttga ccagagcagt
241 gaggtcttag gctctggcaa aacctgacc atccaagtca aagagtttgg agatgctggc
301 cagtacacct gtcacaaaag aggcgaggtt ctaagccatt cgctcctgct gcttcacaaa
361 aaggaagatg gaatttggtc cactgatatt ttaaaggacc agaaagaacc caaaaataag
421 acctttctaa gatcgagggc caagaattat tctggacgtt tcacctgctg gtggctgacg
481 acaatcagta ctgatttgac attcagtgtc aaaagcagca gaggtctctc tgaccccaa
541 ggggtgacgt gcggagctgc tacactctct gcagagagag tcagagggga caacaaggag
601 tatgagtact cagtggagtg ccaggaggac agtgcctgcc cagctgctga ggagagtctg
661 ccatttgagg tcattggtgga tgcggttcac aagctcaagt atgaaaacta caccagcagc
721 ttcttcatca gggacatcat caaacctgac ccaccaaga acttgacgct gaagccatta
781 aagaattctc ggcaggtgga ggtcagctgg gagtacctg acacctggag tactccacat
841 tcctacttct ccttgacatt ctgcgttcag gtccagggca agagcaagag agaaaagaaa
901 gatagagtct tcacggacaa gacctcagcc acggtcatct gccgcaaaaa tgccagcatt
961 agcgtgctgg cccaggaccg ctactatagc tcattcttga gcgaatgggc atctgtgccc
1021 tgcagttagg ttctgatcca ggatgaaaat ttggaggaaa agtggagat attaatgaaa
1081 atgttttaag acacaacgga atagaccaa aaagataatt tctatctgat ttgcttttaa
1141 acgttttttt aggatcacia tgatatcttt gctgtatttg tatagttaga tgctaaatgc
1201 tcattgaaac aatcagctaa tttatgtata gattttccag ctctcaagtt gccatgggcc
1261 ttcattgctat taaatattt aagtaattta tgtatttatt agtatattac tgttatttaa
1321 cgtttgtctg ccaggatgta tggaaatgtt catactctta tgacctgac catcaggatc
1381 agtccctatt atgcaaaatg tgaatttaat tttatttgta ctgacaactt ttcaagcaag
1441 gctgcaagta catcagtttt atgacaatca ggaagaatgc agtgttctga taccagtgcc
1501 atcacatact tgtgatggat gggaacgcaa gagatactta catggaaacc tgacaatgca
1561 aacctgttga gaagatccag gagaacaaga tgctagtctc catgtctgtg aagacttcct
1621 ggagatgggt ttgataaagc aatttagggc cacttacact tctaagcaag tttaatcttt
1681 ggatgcctga attttaaaag ggctagaaaa aaatgattga ccagcctggg aaacataaca
1741 agaccccgct tctacaaaaa aaatttaaaa ttagccaggc gtggtggctc atgcttgtgg
1801 tccagctgt tcaggaggat gaggcaggag gatctcttga gccaggagg tcaaggctat
1861 ggtgagccgt gattgtgcca ctgcatacca gcctagtgga cagaatgaga cctgtctca
1921 aaaaaaaaaa tgattgaaat taaaattcag ctttagcttc catggcagtc ctcaccccca
1981 cctctctaaa agacacagga ggatgacaca gaaacaccgt aagtgtcttg aaggcaaaaa
2041 gatcttaaga ttcaagagag aggacaagta gttatggcta aggacatgaa attgtcagaa
2101 tggcagggtg cttcttaaca gccctgtgag aagcagacag atgcaaagaa aatctggaat
2161 ccctttctca ttgatgtaa tgaacctgat acacaattat gaccagaaaa tatggctcca

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2221 tgaaggtgct acctttaagt aatgtatgtg cgctctgtaa agtgattaca tttgtttcct
 2281 gtttgtttat ttatttattt atttttgcat tctgaggtg aactaataaa aactcttctt
 2341 tgtaatc

(SEQ ID NO: 10)

MCHQQLVISWFLSLVFLASPLVAIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLG
 SGKTLTIQVKEFGDAGQYCHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRF
 TCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSVECQDSACPAEESLPIEV
 MVDVAVHKLKYENYTSFFIRDIIKPDPPKLNQLKPLKNSRQVEVSWEYPTDWTSTPHSYFSLTFCVQVQGK
 SKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSSWSEWASVPCS

15

IFN- γ is a 14-18 kDalton 143 amino acid glycosylated protein that is a potent multifunctional cytokine. As used herein, “interferon-gamma” and “IFN- γ ” refer to IFN- γ protein, biologically active fragments of IFN- γ , and biologically active homologs of “interferon-gamma” and “IFN- γ ”, such as mammalian homologs. As defined herein, biologically active fragments of IFN- γ are fragments that, for example, modulate an immune response to an antigen in a patient who has been administered or otherwise exposed to the antigen. As also defined herein, biologically active fragments or homologs of “interferon-gamma” and “IFN- γ ” include modified IFN- γ protein such that the resulting IFN- γ product has immune response modulating activity similar to the IFN- γ described herein (e.g., the ability to modulate an immune response to an antigen, when administered with the antigen, in a patient, relative to in vivo conditions in the absence of the cytokine or relative to administration of the antigen alone). Biologically active homologs or fragments of “interferon-gamma” also include nucleic acid sequences (e.g., DNA, RNA) and positions thereof, which encode a protein or peptide having the

IFN- γ function or activity described herein (e.g., the ability to modulate an immune response to an antigen in a patient that has been administered or otherwise exposed to the antigen). In addition, the term includes a nucleotide sequence which through the degeneracy of the genetic code encodes a similar peptide gene product as IFN- γ and has the IFN- γ activity described herein. For example, a homolog of “interferon-gamma” and “IFN- γ ” includes a nucleotide sequence which contains a “silent” codon substitution (e.g., substitution of one codon encoding an amino acid for another codon encoding the same amino acid) or an amino acid sequence which contains a “silent” amino acid substitution (e.g., substitution of one acidic amino acid for another acidic amino acid).

An exemplified nucleotide sequence encodes human IFN- γ (Accession No: NM_000639, NCBI database, which is hereby incorporated by reference in its entirety):

A further exemplified nucleotide sequence encodes human IFN- γ (Accession No. NM_000619), NCBI database, which is hereby incorporated by reference in its entirety):

(SEQ ID NO: 11)

1 cacattgttc tgatcatctg aagatcagct attagaagag aaagatcagt taagtccttt
 61 ggacctgac agcttgatac aagaactact gatttcaact tctttggctt aattctctcg
 121 gaaacgatga aatatacaag ttatatcttg gcttttcagc tctgcatcgt tttgggttct
 181 cttggctgtt actgccagga cccatatgta aaagaagcag aaaaccttaa gaaatatttt
 241 aatgcaggtc attcagatgt agcggataat ggaactcttt tottaggcatt tttgaagaat
 301 tggaaagagg agagtgcag aaaaataatg cagagccaaa ttgtctcctt ttacttcaaa
 361 ctttttaaaa actttaaaga tgaccagagc atccaaaaga gtgtggagac catcaaggaa
 421 gacatgaatg tcaagttttt caatagcaac aaaaagaaac gagatgactt cgaaaagctg
 481 actaattatt cggttaactga cttgaatgtc caacgcaaag caatacatga actcatccaa
 541 gtgatggctg aactgtcgcc agcagctaaa acagggaagc gaaaaggagg tcagatgctg
 601 ttctgagggtc gaagagcatc ccagtaatgg ttgtcctgcc tgcaatattt gaattttaaa
 661 tctaaatcta tttattaata tttaacatta tttatatggg gaatatattt ttagactcat
 721 caatcaaata agtatattata atagcaactt ttgtgtaatg aaaatgaata tctattaata
 781 tatgtattat ttataattcc tatatcctgt gactgtctca cttaatcctt tgttttctga
 841 ctaattaggc aaggetatgt gattacaagg ctttatctca ggggccaaat aggcagccaa
 901 cctaagcaag atcccatggg ttgtgtgttt atttcacttg atgatacaat gaacacttat
 961 aagtgaagtg atactatcca gttactgccg gtttgaaaat atgcctgcaa tctgagccag
 1021 tgctttaatg gcattgtcaga cagaactga atgtgtcagg tgacctgat gaaaacatag

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1081 catctcagga gatttcatgc ctgggtgcttc caaatattgt tgacaactgt gactgtaccc
 1141 aaatggaaag taactcattt gttaaaatta tcaatatcta atatatatga ataaagtgt
 1201 agttcacacaac aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa

The corresponding amino acid sequence for human IFN- γ (Accession No. NP_000610), NCBI database, is hereby incorporated by reference in its entirety):

(SEQ ID NO: 12)
 1 MKYTSYILAF QLCIVLGLSG CYQDPYVKE AENLKKYFNA GHSDVADNGT LELGILKNWK
 61 EESDRKIMQS QIVSFYFKLF KNFKDDQSIQ KSVETIKEDM NVKFFNSNKK KRDDFEKLTN
 121 YSVTDLNVQR KAIHELIQVM AELSPAAGTG KRKRSQMLFR GRRASQ

The nucleotide sequences encoding IL-12 and/or IFN- γ used in the subject invention include “homologous” or “modified” nucleotide sequences. Modified nucleic acid sequences will be understood to mean any nucleotide sequence obtained by mutagenesis according to techniques well known to persons skilled in the art, and exhibiting modifications in relation to the normal sequences. For example, mutations in the regulatory and/or promoter sequences for the expression of a polypeptide that result in a modification of the level of expression of a polypeptide according to the invention provide for a “modified nucleotide sequence”. Likewise, substitutions, deletions, or additions of nucleic acids to the polynucleotides of the invention provide for “homologous” or “modified” nucleotide sequences. In various embodiments, “homologous” or “modified” nucleic acid sequences have substantially the same biological or serological activity as the native (naturally occurring) IL-12 and/or IFN- γ peptide. A “homologous” or “modified” nucleotide sequence will also be understood to mean a splice variant of the polynucleotides of the instant invention or any nucleotide sequence encoding a “modified polypeptide” as defined below.

A homologous nucleotide sequence, for the purposes of the present invention, encompasses a nucleotide sequence having a percentage identity with the bases of the nucleotide sequences of between at least (or at least about) 20.00% to 99.99% (inclusive). The aforementioned range of percent identity is to be taken as including, and providing written description and support for, any fractional percentage, in intervals of 0.01%, between 20.00% and 99.99%. These percentages are purely statistical and differences between two nucleic acid sequences can be distributed randomly and over the entire sequence length.

In various embodiments, homologous sequences exhibiting a percentage identity with the bases of the nucleotide sequences of the present invention can have 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identity with the polynucleotide sequences of the instant invention. Homologous nucleic acid sequences and amino acid sequences include mammalian homologs of the human IL-12 and/or IFN- γ nucleic acid sequences and amino acid sequences, including homologs of biologically active fragments, such as biologically active subunits.

Both protein and nucleic acid sequence homologies may be evaluated using any of the variety of sequence comparison

algorithms and programs known in the art. Such algorithms and programs include, but are by no means limited to, TBLASTN, BLASTP, FASTA, TFASTA, and CLUSTALW

(Pearson and Lipman *Proc. Natl. Acad. Sci. USA*, 1988, 85(8):2444-2448; Altschul et al. *J. Mol. Biol.*, 1990, 215(3): 403-410; Thompson et al. *Nucleic Acids Res.*, 1994, 22(2): 4673-4680; Higgins et al. *Methods Enzymol.*, 1996, 266:383-402; Altschul et al. *J. Mol. Biol.*, 1990, 215(3):403-410; Altschul et al. *Nature Genetics*, 1993, 3:266-272).

Identity and similarity of related nucleic acid molecules and polypeptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; York (1988); Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; York (1993); Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Jersey (1994); Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Press (1987); Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; York (1991); and Carillo et al., *SIAM J. Applied Math.*, 48:1073 (1988).

The methods, pharmaceutical compositions, and vectors of the present invention can utilize biologically active fragments of nucleic acid sequences encoding IL-12 and/or IFN- γ . Representative fragments of the polynucleotide sequences according to the invention will be understood to mean any polynucleotide fragment having at least 8 or 9 consecutive nucleotides, preferably at least 12 consecutive nucleotides, and still more preferably at least 15 or at least 20 consecutive nucleotides of the sequence from which it is derived. The upper limit for such fragments is the total number of nucleotides found in the full-length sequence (or, in certain embodiments, of the full length open reading frame (ORF) identified herein).

In other embodiments, fragments can comprise consecutive nucleotides of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, and up to one nucleotide less than the full-length IL-12 and/or IFN- γ coding sequences. In some embodiments, fragments comprise biologically active subunits of IL-12 and/or IFN- γ (e.g., p35 and/or p40 subunit of IL-12), or biologically active fragments of such subunits.

It is also well known in the art that restriction enzymes can be used to obtain biologically active fragments of the nucleic acid sequences, such as those encoding IL-12 and/or IFN- γ . For example, Bal31 exonuclease can be conveniently used for time-controlled limited digestion of DNA (commonly referred to as "erase-a-base" procedures). See, for example, Maniatis et al. [1982] *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York; Wei et al. [1983] *J. Biol. Chem.* 258:13006-13512.

The methods of the subject invention also contemplate the administration of cells that have been genetically modified to produce both IL-12 and IFN- γ , or biologically active fragments thereof. Such genetically modified cells can be administered alone or in combinations with different types of cells. Thus, genetically modified cells of the invention can be co-administered with other cells, which can include genetically modified cells or non-genetically modified cells. Genetically modified cells may serve to support the survival and function of the co-administered cells, for example.

The term "genetic modification" as used herein refers to the stable or transient alteration of the genotype of a cell of the subject invention by intentional introduction of exogenous nucleic acids by any means known in the art (including for example, direct transmission of a polynucleotide sequence from a cell or virus particle, transmission of infective virus particles, and transmission by any known polynucleotide-bearing substance) resulting in a permanent or temporary alteration of genotype. The nucleic acids may be synthetic, or naturally derived, and may contain genes, portions of genes, or other useful polynucleotides in addition to those encoding IL-12 and IFN- γ , or biologically active fragments thereof. A translation initiation codon can be inserted as necessary, making methionine the first amino acid in the sequence. The term "genetic modification" is not intended to include naturally occurring alterations such as that which occurs through natural viral activity, natural genetic recombination, or the like. The genetic modification may confer the ability to produce IL-12 and IFN- γ , or biologically active fragments thereof, wherein the cell did not previously have the capability, or the modification may increase the amount of IL-12 and IFN- γ produced by the cell, e.g., through increased expression.

Exogenous nucleic acids and/or vectors encoding IL-12 and/or IFN- γ , or biologically active fragments thereof, can be introduced into a cell by viral vectors (retrovirus, modified herpes virus, herpes virus, adenovirus, adeno-associated virus, and the like) or direct DNA transfection (lipofection, calcium phosphate transfection, DEAE-dextran, electroporation, and the like), microinjection, cationic lipid-mediated transfection, transduction, scrape loading, ballistic introduction and infection (see, for example, Sambrook et al. [1989] *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

Preferably, the exogenous nucleic acid sequence encoding IL-12 and/or IFN- γ , or biologically active fragments thereof, is operably linked to a promoter sequence that permits expression of the nucleic acid sequence in a desired tissue within the patient. The promoters can be inducible or tissue specific as necessary.

The genetically modified cell may be chosen from eukaryotic or prokaryotic systems, for example bacterial cells (Gram negative or Gram positive), yeast cells, animal cells, plant cells, and/or insect cells using baculovirus vectors. In some embodiments, the genetically modified cell for expression of the nucleic acid sequences encoding IL-12 and/or IFN- γ , or biologically active fragments thereof, are human or non-human mammal cells.

Standard molecular biology techniques known in the art and not specifically described were generally followed as in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York (1989), and in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Md. (1989) and in Perbal, *A Practical Guide to Molecular Cloning*, John Wiley & Sons, New York (1988), and in Watson et al., *Recombinant DNA*, Scientific American Books, New York and in Birren et al. (eds) *Genome Analysis: A Laboratory Manual Series*, Vols. 1-4 Cold Spring Harbor Laboratory Press, New York (1998) and methodology as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659; and 5,272,057; and incorporated herein by reference. Polymerase chain reaction (PCR) was carried out generally as in PCR Protocols: A Guide To Methods And Applications, Academic Press, San Diego, Calif. (1990). In situ (In-cell) PCR in combination with Flow Cytometry can be used for detection of cells containing specific DNA and mRNA sequences (Testoni et al., *Blood*, 1996, 87:3822.)

Materials and Methods

Animals.

Female B6D2 μ l mice, 6 to 8 weeks old, from Jackson Laboratory (Bar Harbor, Me.) were maintained in pathogen-free conditions at the animal center at the James A. Haley Veterans Hospital. All procedures were reviewed and approved by the committee on animal research at the James A. Haley VA Medical Center and the University of South Florida College of Medicine.

Vaccination Protocol.

Four groups of naive mice (n=12) were intramuscularly vaccinated 3 times at intervals of 2 days, each in its right quadriceps muscle, with 100 μ g of pIFN- γ , 100 μ g of pIL-12, or a mixture of pIL-12 and pIFN- γ (50 μ g of each), along with subcutaneous injection of 50 μ g per mouse of crude KBG extract given at the back of the animal. Control mice each received 100 μ g of pcDNA3.1 plasmid and 50 μ g of KBG allergen extract. Seven days after the last DNA and KBG vaccinations, control and experimental groups of mice were immunized intraperitoneally with 10 μ g of KBG allergen and 1 mg of alum.

Construction of the pIL-12 and pIFN- γ Plasmids.

To clone murine IL-12, the IL-12 p40 subunit was amplified from a mouse cDNA library as an NheI-XhoI cassette through use of the following set of primers: forward primer 5'-dCCA GGC AGC TAG CAG CAA AGC AA-3' (SEQ ID NO:1) and reverse primer 5'-dTCC CTC GAG GCA TCC TAG GAT CGG AC-3' (SEQ ID NO:2). The amplified product was ligated to the mammalian expression vector pcDNA 3.1 (INVITROGEN, San Diego, Calif.) at the corresponding sites. The resulting plasmid, pcP40, was used as the template to amplify the p40 subunit and bovine growth hormone (BGH) poly A sequences derived from the pcDNA 3.1 vector as an HindIII-KpnI cassette and ligated to pcDNA 3.1 at the corresponding sites to generate the plasmid pc40. The following primers were used: 5'-dACC CAA GCT TGC TAG CAG CAA A-3' (SEQ ID NO:3) and 5'-dGAA GCC ATA GAG GGT ACC GCA TC-3' (SEQ ID NO:4). Through use of forward primer 5'-dTGC GGA TCC AGC ATG TGT CAA T-3' (SEQ ID NO:5) and reverse primer 5'-dGCA GAG GGC CTC GAG CTT TCA G-3' (SEQ ID NO:6), the p35 subunit of murine IL-12 was amplified as a BamHI-XhoI fragment and cloned into pcDNA3.1 to generate the pcP35 vector. With pcP35 used as a template, the cytomegalovirus (CMV) promoter and p35 subunit were as an EcoRI-EcoRV cassette and

ligated to the corresponding site in the vector pc40. The resulting plasmid, pIL-12, had each of the 2 subunits of IL-12 under a separate CMV promoter and a BGH gene poly A sequence. Murine IFN- γ was cloned in pcDNA3.1; the construction of this plasmid has been described elsewhere (Kumar, M. et al., *Vaccine*, 1999, 18:558-567). Large-scale plasmid preparation was performed through use of a QIAGEN kit (QIAGEN, Valencia, Calif.).

Immunohistochemistry.

Groups of naive mice were vaccinated intramuscularly 3 times with 100 μ g of pIFN- γ , pIL-12, or pcDNA3.1 control vector at intervals of 2 days each. Seven days after the last vaccination, the mice were killed and their right quadriceps muscles were removed and subjected to paraffin embedding, as described previously (Kumar, M. et al., *Vaccine*, 1999, 18:558-567). Immunostaining for IFN- γ and IL-12 was performed through use of polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, Calif.) against murine IFN- γ and the murine IL-12 p40 subunit, as described previously (Matsuse, H. et al., *J. Immunol.*, 2000, 164:6583-6592).

Splenocyte Culture and Assay for Cytokines.

Seven days after the KBG immunization, one half of the animals in each group were killed and their spleens removed aseptically. Single-cell suspensions were cultured with 1 mg/mL KBG allergen for 48 hours. Supernatants were collected from the cultures, and the production of IFN- γ , IL-2, and IL-4 was determined by ELISA (R&D Systems, Minneapolis, Minn.), according to the manufacturer's instructions.

Antibody Assays.

Mice were bled 21 days after KBG immunization, and their sera were collected. Total IgE and antigen-specific IgG 1 and IgG2a were estimated by ELISA through use of purified antimouse mAbs (PHARMINGEN, San Diego, Calif.). Microtiter plates (COSTAR, Cambridge, Mass.) were coated overnight with 0.1 μ g per well of purified KBG allergen in a bicarbonate buffer (0.05 mol/L, pH 9.6) or antimouse IgE mAbs. Wells were washed with washing buffer (0.5% Tween-20 in PBS, pH 7.4) and blocked with 200 μ L per well of PBS (pH 7.4) containing 1% BSA for 1 hour at 37° C. After 3 washes, serum samples were added at 100 μ L per well and incubated for 2 hours at 37° C. After washing, biotinylated antimouse IgG1, IgG2a, and IgE antibodies were added to each well and incubated at 37° C. for 1 hour. Streptavidin-peroxidase conjugate (1:10,000 dilution, SIGMA CHEMICALS, St Louis, Mo.) was added after washing, and the wells were further incubated at 37° C. for 1 hour. Finally, the plates were washed and color was developed by the addition of substrate tetramethyl benzidine (PHARMINGEN) at room temperature for 30 minutes. The reaction was stopped and the absorbance read at 450 nm through use of an automated ELISA reader.

Pulmonary Function.

To assess pulmonary function, naive mice (n=4) were vaccinated 3 times as described in connection with the vaccination protocol. Seven days after the last cytokine pDNA and KBG vaccinations, animals were sensitized intraperitoneally with 10 μ g of KBG allergen and 1 mg of alum. Ten days later, these mice were challenged intranasally with 50 μ g of KBG allergen once daily for 3 consecutive days. Airway responsiveness was measured with a whole-body plethysmograph (BUXCO ELECTRONICS, Troy, N.Y.) in conscious, unrestrained mice at 24 hours after allergen challenge; response to methacholine was expressed as enhanced pause, as previously described (Matsuse, H. et al., *J. Immunol.*, 2000, 164: 6583-6592).

Histologic Analysis.

Mice were killed with an overdose (0.6 g/kg) of pentobarbital (NEMBUTAL, Abbott Laboratories, North Chicago, Ill.) 24 hours after the final allergen challenge, and lung sections were subjected to paraffin embedding. Lung inflammation was assessed after the sections were stained with hematoxylin and eosin; this was followed by scoring for severity of inflammation on a scale of 0 to 3 (0=minimum degree of inflammation; 3=the maximum), as described previously (Kumar, M. et al., *Vaccine*, 1999, 18:558-567). Pathologic scores were expressed as means \pm SDs. The slides were coded and scored in a blinded fashion twice each by 3 different individuals. Intraobserver variation was <5%.

Statistical Analysis.

Pairs of groups were compared through use of Student t tests. Differences between groups were considered significant at P<0.05. Values for all measurements are expressed as means \pm SDs.

Example 1

Plasmid Constructs and Expression of IL-12 and IFN- γ

The 2 subunits of murine IL-12, p35 and p40, were cloned into the same pcDNA3.1 vector. The cloning strategy was such that each subunit was under the transcriptional control of an individual CMV immediate-early promoter and also had its own BGH poly A sequences derived from the vector pcDNA3.1. Mice given pIFN- γ or pIL-12 exhibited expression of the IFN- γ or IL-12 p40 subunit, respectively, in their muscle, as observed by immunohistochemical staining of the muscle tissues (FIGS. 1A and 1C). No immunostaining was observed in a control group of mice that received the empty vector pcDNA3.1 (FIGS. 1B and 1D). The results of RT-PCR analyses of muscle mRNAs revealed that mice given either pIFN- γ or pIL-12 (FIG. 1E, lane 2 and lane 4), but not the control group of mice (FIG. 1E, lane 1 and lane 3), expressed IFN- γ and IL-12 p40 subunit-specific mRNAs, respectively.

Example 2

Cytokine Genetic Adjuvants Inhibit Production of IgE Antibodies and Enhanced Production of IgG2a Antibodies

Four groups of mice (n=6) were vaccinated 3 times, as described in connection with the vaccination protocol. Sera were collected on day 21 after KBG immunization for an antibody assay. As shown in FIGS. 2A and 2B, mice given cytokine plasmid(s) as an adjuvant exhibited significantly lower total IgE levels than control mice (P<0.01). The group of mice given both pIL-12 and pIFN- γ constructs revealed a significantly lower amount of total IgE than mice given pIL-12 (P<0.01) or pIFN- γ (P<0.05) alone (FIG. 2B). There was also an increase in antigen-specific IgG2a levels (P<0.01) in mice given pIFN- γ plus pIL-12 as an adjuvant in comparison with the control mice and the mice receiving either plasmid alone (FIG. 2A). However, no significant difference was observed for antigen-specific IgG 1 antibody levels among the cytokine pDNA-vaccinated and control groups of mice (FIG. 2A). These results indicate that administration of cytokine pDNA adjuvants along with allergen vaccines resulted in a shift in the antibody production from the IgE type to the IgG2a type.

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Example 3

Cytokine Genetic Adjuvants Alter the Cytokine Profiles of Splenocytes

Four groups of mice (n=6) were vaccinated 3 times as described in connection with the vaccination protocol. Spleens were removed on day 7, after KBG immunization, and cultured in vitro for assessment of T_H1-like cytokines IL-2 and IFN- γ or T_H2-like cytokine IL-4. Mice given cytokine pDNA adjuvants produced more T_H1-like cytokines than did the controls (FIGS. 3A-3C). Mice given combined pIFN- γ and pIL-12 produced higher amounts of IFN- γ (194.64 pg/mL) than mice given pIFN- γ (100.14 pg/mL) or pIL-12 (111.87 pg/mL) alone. Control mice produced only 11.71 pg/mL IFN- γ (FIG. 3A). Mice vaccinated with pDNA cytokine(s) adjuvant produced more IL-2 than did control mice. IL-2 levels after vaccinations were as follows: pIFN- γ , 73.68 pg/mL; pIL-12, 128.43 pg/mL; pIFN- γ plus pIL-12, 202.57 pg/mL; and pcDNA3.1 (control), 51.88 pg/mL (FIG. 3A). In contrast, control mice, which were vaccinated only with the empty vector plasmid, produced more IL-4 (37.55 pg/mL) than the groups injected with the pDNA cytokine adjuvant, which produced 9.71 pg/mL (pIFN- γ plus pIL-12), 13.05 pg/mL (pIFN- γ) and 17.99 pg/mL (pIL-12) of IL-4 (FIG. 3B). No significant difference was observed in IL-4 production among the pIFN- γ , pIL-12, and pIFN- γ plus pIL-12 treatment groups.

To examine the dominant pattern of cytokine responses, IFN- γ :IL-4 and IL-2:IL-4 ratios were compared among different groups of mice (FIG. 3C). The IFN- γ :IL-4 ratios in the mice vaccinated with pIFN- γ , pIL-12, and pIFN- γ plus pIL-12 as adjuvants were 7.48, 6.33, and 20.86, respectively, whereas the empty pcDNA3.1-vaccinated control exhibited a

ratio of only 0.31. The ratios of IL-2:IL-4 in pIFN- γ , IL-12, pIFN- γ plus IL-12, and pcDNA3.1 controls were 7.50, 7.32, 21.71, and 1.4, respectively. These results indicate that the net cytokine balance shifted in favor of the T_H1-like response in cytokine plasmid-vaccinated mice; however, this shift was greater in the group vaccinated with the adjuvant that was a combination of pIFN- γ and pIL-12 plasmid DNAs.

Example 4

Cytokine Genetic Adjuvants Prevent the Development of Airway Hyperresponsiveness in Allergen-Sensitized and -Challenged Mice

Mice (n=4) were vaccinated, sensitized, and challenged as described in connection with pulmonary function. The air-

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ways of control mice that received the empty vector as vaccine adjuvant were significantly more reactive to 50 mg/mL of methacholine than those of the mice that received pIFN- γ and/or pIL-12, as shown in FIG. 4. Mice vaccinated with pIFN- γ plus pIL-12 exhibited the least reactivity to the inhaled methacholine challenge in comparison with the pcDNA3.1 (P<0.01), pIFN- γ , and pIL-12 groups (P<0.05). These results suggest that the vaccination of mice with combined pIFN- γ and pIL-12 as an adjuvant significantly reduces airway reactivity.

Example 5

Combination of IFN- γ and IL-12 Plasmid DNAs Prevents Lung Inflammation in the Allergen-Sensitized Mice

Mice (n=4) were vaccinated, sensitized, and challenged as described in connection with pulmonary function. Lung inflammation was examined 24 hours after the final allergen challenge. Representative pathologic features are shown in FIGS. 5A-5D. The group of mice receiving the combined constructs as an adjuvant (FIG. 5D) exhibited less epithelial damage and less infiltration of mononuclear cells and polymorphs in the interstitial and peribronchovascular region than was seen in the control group (FIG. 5A) and the other vaccinated groups (FIGS. 5B and 5C). A semi-quantitative analysis using a scoring system for inflammatory cells in the lung is shown in Table 1. Groups of mice that received pIFN- γ , pIL-12, or a combination of the two exhibited reduced pulmonary inflammation in comparison with the empty vector (pcDNA3.1) control. The group of mice that received the combination of pIFN- γ and pIL-12 showed significantly less (P<0.05) pulmonary inflammation than the pIFN- γ and pIL-12 groups. No statistically significant difference was found between the pIFN- γ and pIL-12 groups.

TABLE 1

Quantification of pulmonary inflammation in mice after allergen vaccination with various cytokine adjuvants				
Pathology	pcDNA3.1	Adjuvants pIFN- γ	pIL-12	pIFN- γ + pIL-12
Epithelial damage	2.8 \pm 0.12	2.23 \pm 0.23*	2.3 \pm 0.16*	1.36 \pm 0.48**†
Interstitial-alveolar infiltrate	2.73 \pm 0.20	2.4 \pm 0.25*	2.56 \pm 0.42	1.76 \pm 0.36**†
Peribronchovascular infiltrate	2.83 \pm 0.26	2.16 \pm 0.23*	2.16 \pm 0.32*	1.26 \pm 0.20**†

Each value represents the mean \pm SD of 5 fields from 6 individual lung sections from each mouse in a group (n = 4). Values were considered significant when the P value was less than .05**†. Statistical differences are indicated as follows:

*in comparison with pcDNA3.1 control;

†in comparison with pIFN- γ control;

‡in comparison with pIL-12 control.

Previously, it was shown in a mouse model that effective parenteral vaccination of mice with grass allergens required subcutaneous injection of a high dose (250 μ g to 1 mg per mouse) of allergens (Cao, Y. et al., *Immunology*, 1997, 90:46-51). By corollary, an even higher dose of allergen mixture would be required to induce an effective immune deviation from an allergic response. The results of this study demonstrate that in a mouse, a substantially lower dosage (50 μ g) of allergens, when administered along with a pDNA cytokine(s) adjuvant, can induce effective immune deviation and a protective airway response in comparison with allergens alone. Furthermore, a comparison of pIFN- γ and/or pIL-12 as adjuvants indicates that the combination of pIFN- γ and pIL-12 is a more effective adjuvant than either of these plasmids alone. Although the effect of these adjuvants in mice with an established allergic response was not examined, a number of stud-

ies have reported the effectiveness of INF- γ and of IL-12 in modulating established allergic responses (Li, X. M. et al., *J Immunol*, 1996, 157:3216-3219; Hogan, S. P. et al., *Eur J Immunol*, 1998, 28:413-423; Dow, S. W. et al., *Hum Gene Ther*, 1999, 10:1905-1914; Maecker, H. T. et al., *J Immunol*, 2001, 166:959-965). The results of the study, therefore, have significant implications for "time-honored" AIT, which might be rendered more safe and effective by including these cytokine plasmids as adjuvants.

A major finding of this study is that a vaccine adjuvant comprising both pIFN- γ and pIL-12 inhibited total IgE levels in mice with a concomitant increase of allergen-specific IgG2a antibody production. The level of IgE synthesis in mice vaccinated with a cocktail adjuvant was significantly ($P < 0.001$) more suppressed than that of the levels obtained by vaccination with pIFN- γ or pIL-12 alone as an adjuvant. Both systemic and local IFN- γ gene delivery has been shown to decrease serum IgE levels (Dow, S. W. et al., *Hum Gene Ther*, 1999, 10:1905-1914), whereas the role of the IL-12 protein as an adjuvant on serum IgE levels has been controversial (Kips, J. C. et al., *Am J Respir Crit Care Med*, 1996, 153:535-539; Brusselle, G. G. et al., *Am. J Respir Cell Mol Biol*, 1997, 17:767-771; Sur, S. et al., *J Immunol*, 1996, 157:4173-4180; Yoshimoto, T. et al., *Proc Natl Acad Sci USA*, 1997, 94:3948-3953). The results of this study indicate that an adjuvant comprising the IFN- γ and IL-12 plasmids enhances the production of IFN- γ . This finding is in agreement with a report that mice vaccinated with IL-12 exhibited enhanced local IFN- γ production (Schwarze, J. et al., *J Allergy Clin Immunol*, 1998, 102:86-93).

The deviation of the cytokine production profile from T_H2 -like to T_H1 -like is a significant marker of the effectiveness of a vaccine. Analysis of the cytokine pattern of mice after vaccination with allergens and different plasmid adjuvants revealed that the group of mice receiving pIFN- γ plus pIL-12 as the adjuvant had a more profound T_H1 -like effect—i.e., it produced higher levels of IFN- γ and IL-2 than either of the cytokine plasmids alone. Analysis of the IL-4 expression from the splenocytes of different groups of mice revealed no significant difference among the groups of mice vaccinated with pIFN- γ , pIL-12, or a combination of the two. A predominance of the T_H1 -like response seen with cytokine adjuvants is consistent with the finding of Hogan et al. (Hogan, S. P. et al., *Eur J Immunol*, 1998, 28:413-423), who showed, using the vaccinia virus as a gene delivery vehicle, that IL-12 gene transfer to murine lung inhibited T_H2 cytokine production, which was mediated through the production of IFN- γ . Because allergic individuals produce limited amounts of IFN- γ , an adjuvant providing the expression of both IFN- γ and IL-12 induces a more effective T_H1 -like response.

In patients with allergic asthma, it was shown that airway hyperresponsiveness correlates with the severity of disease and is therefore a major clinical feature of allergic asthma. A direct instillation into the respiratory tract or an intraperitoneal administration of either rIL-12 or rIFN- γ inhibits allergic airway inflammation and in some cases suppresses airway hyperresponsiveness (Gavett, S. H. et al., *J Exp Med*, 1995, 182:1527-1536; Iwamoto, I. et al., *J Exp Med*, 1993, 177:573-576). However, these protocols require a regular or daily dosing over periods ranging from 5 to 13 days to produce significant protective effects. Furthermore, gene transfer studies using IFN- γ and IL-12 have been shown to suppress airway hyperreactivity (Li, X. M. et al., *J Immunol*, 1996, 157:3216-3219; Hogan, S. P. et al., *Eur J Immunol*, 1998, 28:413-423). The results of this investigation indicate that allergen vaccine formulations that include allergens and pIFN- γ , pIL-12, or a combination of both cytokines as an

adjuvant significantly decrease airway hyperresponsiveness and that a combination of pIFN- γ and pIL-12 induces the highest reduction in airway responsiveness. There appears to be a synergistic effect when both cytokine plasmids (pIFN- γ and pIL-12) are administered to the mice. A parenteral administration of only 50 μ g of allergen-induced massive cellular infiltration and airway obstruction in the lung tissue of mice in the present study. However, allergens given with pIFN- γ , pIL-12, or a combination as an adjuvant limited not only the cellular infiltration but also epithelial cell damage. Consistent with previous observations, either pIFN- γ or pIL-12 as an adjuvant decreased eosinophilic cellular infiltration (Li, X. M. et al., *J Immunol*, 1996, 157:3216-3219; Hogan, S. P. et al., *Eur J Immunol*, 1998, 28:413-423), whereas treatment with a combination of pIFN- γ and pIL-12 as an adjuvant was most effective.

Not wishing to be bound by theory, one mechanism for the effectiveness of pIFN- γ and pIL-12 as an adjuvant may be that CpG sequences, which are present in the backbone plasmid pcDNA3.1, nonspecifically contribute to the endogenous IL-12/IFN- γ production (Roman, M. et al., *Nat Med*, 1997, 3:849-854). One possibility is that CpG sequences present in the backbone plasmid, pcDNA3.1, nonspecifically contribute to the endogenous IL-12/IFN- γ production (Roman, M. et al., *Nat Med*, 1997, 3:849-854). However, this is unlikely, because all groups received an equal dose of plasmid and mice receiving both pIFN- γ and pIL-12 received an adjusted dose (50 μ g each, for a total of 100 μ g). The empty vector, which also contained CpGs, had no cytokine-inducing or immunomodulatory effect. Furthermore, the higher effectiveness of combined pIFN- γ and pIL-12 in comparison with either of the plasmids alone (100 μ g) might be attributed to the CpG motifs in the coding sequences; however, the cDNA inserts are mammalian sequences, which have a low frequency of CpG dinucleotides, are mostly methylated, and do not have immunostimulatory activity (Hemmi, H. et al., *Nature*, 2000, 408:740-750). Nonetheless, a search for a canonical hexamer purine-purine-CG-pyrimidine-pyrimidine as a possible core CpG motif led to the identification of GACGTT, GGCGTC, GGCGTT sequences in IL-12, and AACGCT, AGCGCT sequences in IFN- γ . However, whether these sequences in the mouse cDNA are capable of binding to the mouse Toll-like receptor 9 and cause immune stimulation, like the CpG motifs present in the bacterial DNA, remains to be investigated (Hemmi, H. et al., *Nature*, 2000, 408:740-750).

Alternatively, the synergy between pIFN- γ and pIL-12 on the induction of a T_H1 -dominant state and the inhibition of airway inflammation may be in their cellular mechanism of action. First, although IL-12 is the primary determinant of the T_H1 differentiation, IFN- γ endogenously synthesized during the priming of naive CD4-positive T cells both accelerates and enhances the T_H1 differentiating effects of IL-12 (Wenner, C. A. et al., *J Immunol*, 1996, 156:1442-1447; Bradley, L. M. et al., *J Immunol*, 1996, 157:1350-1358). Thus, IFN- γ presumably synergizes with T-cell receptor engagement and induces the expression of functional IL-12 receptors on naive T cells. This idea is consistent with the report that the presence of IFN- γ and IFN- α in cultures enhanced IL-12R β 2 expression on CD4-positive and CD8-positive T cells and that the enhancing effect of IFN- γ was independent of endogenous IL-12 or IFN- α (Wu, C. Y. et al., *Eur J Immunol*, 2000, 30:1364-1374). Second, the highest amount of IL-2 produced by a combined vaccination with pIFN- γ and pIL-12 might synergize with IFN- γ effects and contribute to a more effective T_H1 -like response. IL-2 regulates expression of the IL-12 β 2 receptor on natural killer cells (Wu, C. Y. et al., *Eur*

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J Immunol, 2000, 30:1364-1374). It is to be noted however, that these 2 possibilities are not mutually exclusive. Irrespective of the mechanism involved, the results lead to the conclusion that in a mouse model of grass allergy, the administration of cytokine plasmids encoding IFN- γ and IL-12 as an adjuvant enhances the therapeutic effectiveness of grass allergen vaccines.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated

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by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

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aaaaaaaaa tgattgaaat taaattcag ctttagcttc catggcagtc ctcaccccca	1980
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gatcttaaga ttcaagagag aggacaagta gttatggcta aggacatgaa attgtcagaa	2100
tggcaggtgg cttcttaaca gcctgtgag aagcagacag atgcaaagaa aatctggaat	2160
ccctttctca ttagcatgaa tgaacctgat acacaattat gaccagaaaa tatggctcca	2220
tgaaggtgct acttttaagt aatgtatgtg cgctctgtaa agtgattaca tttgtttcct	2280
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<211> LENGTH: 328

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

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20 25 30	
Val Glu Leu Asp Trp Tyr Pro Asp Ala Pro Gly Glu Met Val Val Leu	
35 40 45	
Thr Cys Asp Thr Pro Glu Glu Asp Gly Ile Thr Trp Thr Leu Asp Gln	
50 55 60	
Ser Ser Glu Val Leu Gly Ser Gly Lys Thr Leu Thr Ile Gln Val Lys	
65 70 75 80	
Glu Phe Gly Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Val	
85 90 95	
Leu Ser His Ser Leu Leu Leu Leu His Lys Lys Glu Asp Gly Ile Trp	
100 105 110	
Ser Thr Asp Ile Leu Lys Asp Gln Lys Glu Pro Lys Asn Lys Thr Phe	

-continued

115	120	125
Leu Arg Cys Glu Ala Lys Asn Tyr Ser Gly Arg Phe Thr Cys Trp Trp 130 135 140		
Leu Thr Thr Ile Ser Thr Asp Leu Thr Phe Ser Val Lys Ser Ser Arg 145 150 155 160		
Gly Ser Ser Asp Pro Gln Gly Val Thr Cys Gly Ala Ala Thr Leu Ser 165 170 175		
Ala Glu Arg Val Arg Gly Asp Asn Lys Glu Tyr Glu Tyr Ser Val Glu 180 185 190		
Cys Gln Glu Asp Ser Ala Cys Pro Ala Ala Glu Glu Ser Leu Pro Ile 195 200 205		
Glu Val Met Val Asp Ala Val His Lys Leu Lys Tyr Glu Asn Tyr Thr 210 215 220		
Ser Ser Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn 225 230 235 240		
Leu Gln Leu Lys Pro Leu Lys Asn Ser Arg Gln Val Glu Val Ser Trp 245 250 255		
Glu Tyr Pro Asp Thr Trp Ser Thr Pro His Ser Tyr Phe Ser Leu Thr 260 265 270		
Phe Cys Val Gln Val Gln Gly Lys Ser Lys Arg Glu Lys Lys Asp Arg 275 280 285		
Val Phe Thr Asp Lys Thr Ser Ala Thr Val Ile Cys Arg Lys Asn Ala 290 295 300		
Ser Ile Ser Val Arg Ala Gln Asp Arg Tyr Tyr Ser Ser Ser Trp Ser 305 310 315 320		
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<210> SEQ ID NO 11

<211> LENGTH: 1240

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

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gaaacgatga aatatacaag ttatatcttg gcttttcagc tctgcacgt tttgggttct     180
cttggtgtgt actgccagga cccatatgta aaagaagcag aaaaccttaa gaaatatttt     240
aatgcaggtc attcagatgt agcggataat ggaactcttt tcttaggcat ttgaagaat      300
tggaagagg agagtgcag aaaaataatg cagagccaaa ttgtctcctt ttacttcaaa     360
ctttttaaaa actttaaaga tgaccagagc atccaaaaga gtgtggagac catcaaggaa     420
gacatgaatg tcaagttttt caatagcaac aaaaagaaac gagatgactt cgaaaagctg     480
actaattatt cggtaactga cttgaatgtc caacgcaaag caatacatga actcatccaa     540
gtgatggctg aactgtcgcc agcagctaaa acagggaagc gaaaaaggag tcagatgctg     600
tttcgaggtc gaagagcatc ccagtaatgg ttgtcctgcc tgcaatatTT gaattttaaa     660
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caatcaaata agtatttata atagcaactt ttgtgtaatg aaaatgaata tctattaata     780
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ctaattaggc aaggctatgt gattacaagg ctttatctca ggggccaaact aggcagccaa     900
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catctcagga gatttcatgc ctgggtgcttc caaatattgt tgacaactgt gactgtaccc 1140
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<210> SEQ ID NO 12
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 12

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Asn Leu Lys Lys Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn
35          40          45
Gly Thr Leu Phe Leu Gly Ile Leu Lys Asn Trp Lys Glu Glu Ser Asp
50          55          60
Arg Lys Ile Met Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe
65          70          75          80
Lys Asn Phe Lys Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile
85          90          95
Lys Glu Asp Met Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg
100         105         110
Asp Asp Phe Glu Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val
115         120         125
Gln Arg Lys Ala Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser
130         135         140
Pro Ala Ala Lys Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Arg
145         150         155         160
Gly Arg Arg Ala Ser Gln
165

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What is claimed is:

1. A method for modulating an immune response, comprising administering host cells and an antigen to a mammal, wherein the host cells have been obtained from the mammal and have been genetically modified with an expression vector to produce, or increase production of, p35 and p40 subunits of IL-12, and IFN- γ , by the host cells, wherein the expression vector comprises a nucleic acid encoding the p35 and p40 subunits of IL-12 and IFN- γ operably linked to a promoter, wherein the p35 and p40 subunits of IL-12 and IFN- γ are secreted by the administered host cells in the mammal, and wherein said administering of the host cells and the antigen result in an increase of IFN- γ and IL-2 production, an increase of IgG2a specific to the antigen, a decrease of IL-4 production, and reduced serum IgE in the mammal.

2. The method of claim 1, wherein the mammal is a human, wherein the IL-12 is human IL-12, and wherein the IFN- γ is human IFN- γ .

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3. The method of claim 2, wherein the p35 subunit comprises the amino acid sequence of SEQ ID NO:8, and wherein the p40 subunit comprises the amino acid sequence of SEQ ID NO:10.

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4. The method of claim 2, wherein the IFN- γ comprises the amino acid sequence of SEQ ID NO:12.

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5. The method of claim 1, wherein the antigen is selected from the group consisting of a protein, peptide, glycoprotein, carbohydrate, lipid, glycolipid, hapten conjugate, recombinant nucleotides, killed or attenuated organism, toxin, toxoid, and organic molecule.

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6. The method of claim 1, wherein the host cells do not express endogenous p35 and p40 subunits of IL-12 and IFN- γ .

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7. The method of claim 1, wherein the expression vector comprises a DNA plasmid or viral vector.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 8,603,458 B2
APPLICATION NO. : 12/476896
DATED : December 10, 2013
INVENTOR(S) : Shyam S. Mohapatra et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification

Column 1,

Line 64, "(Paironchi, P." should read --(Parronchi, P.--

Column 13,

Lines 34-35, "positions thereof" should read --portions thereof--

Column 15,

Line 12, (SEQ ID NO: 12), "LELGILKNWK" should read --LFLGILKNWK--

Column 18,

Line 25, "Female B6D2 µl mice" should read --Female B6D2F1 mice--

Column 19,

Line 9, "Immunohistochemisty" should read --Immunohistochemistry--

Line 32, "IgG 1 and" should read --IgG1 and--

Column 20,

Line 62, "IgG 1 antibody" should read --IgG1 antibody--

Signed and Sealed this
Thirteenth Day of May, 2014



Michelle K. Lee
Deputy Director of the United States Patent and Trademark Office