Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2494888 C 2009/03/31

(11)(21) **2 494 888**

(12) **BREVET CANADIEN**
CANADIAN PATENT

(13) **C**

(86) Date de dépôt PCT/PCT Filing Date: 2003/08/06
(87) Date publication PCT/PCT Publication Date: 2004/04/29
(45) Date de délivrance/Issue Date: 2009/03/31
(85) Entrée phase nationale/National Entry: 2005/02/04
(86) N° demande PCT/PCT Application No.: US 2003/024625
(87) N° publication PCT/PCT Publication No.: 2004/034966
(30) Priorité/Priority: 2002/08/06 (US60/401,652)

(51) Cl.Int./Int.Cl. *A61K 48/00* (2006.01), *C12N 15/87* (2006.01), *C12N 5/00* (2006.01), *C12N 15/12* (2006.01), *C12N 15/60* (2006.01), *C12N 15/85* (2006.01)

(72) Inventeurs/Inventors:
ESCHER, ALAN P., US;
LI, FENGCHUN, US

(73) Propriétaire/Owner:
LOMA LINDA UNIVERSITY, US

(74) Agent: GOWLING LAFLEUR HENDERSON LLP

(54) Titre : SUBSTANCES POUR PREVENIR ET TRAITER LES MALADIES AUTO-IMMUNES
(54) Title: SUBSTANCES FOR PREVENTING AND TREATING AUTOIMMUNE DISEASES

(57) Abrégé/Abstract:
A substance for preventing, delaying the onset of, or treating one or more than one autoimmune disease, the substance comprising a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for the autoimmune disease. A method for preventing, delaying the onset of or treating an autoimmune disease in a patient comprising selecting a patient who is susceptible to developing the autoimmune disease, who is developing the autoimmune disease or who has the autoimmune disease and administering to the patient one or more than one dose of a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for an autoimmune disease, or comprising a polynucleotide sequence encoding the adenoviral protein E3-GP19k, or comprising a polynucleotide sequence encoding ΔBCL-2.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 April 2004 (29.04.2004)

PCT

(10) International Publication Number
WO 2004/034966 A3

(51) International Patent Classification[7]: C12N 5/00, 15/00, 15/87, A61K 48/00

(21) International Application Number: PCT/US2003/024625

(22) International Filing Date: 6 August 2003 (06.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/401,652 6 August 2002 (06.08.2002) US

(71) Applicant *(for all designated States except US)*: **LOMA LINDA UNIVERSITY** [US/US]; 2488 Prospect Street, Loma Linda, CA 92354 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **ESCHER, Alan, P.** [US/US]; 463 Jefferson Street, Redlands, CA 92374 (US). **LI, Fengchun** [CN/US]; 24530 University Avenue, Apt. 10, Loma Linda, CA 92354-2732 (US).

(74) Agents: **FARAH, David, A.** et al.; Sheldon & Mak, PC, 225 South Lake Avenue, 9th Floor, Pasadena, CA 91101 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
8 July 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/034966 A3

(54) Title: SUBSTANCES FOR PREVENTING AND TREATING AUTOIMMUNE DISEASES

(57) Abstract: A substance for preventing, delaying the onset of, or treating one or more than one autoimmune disease, the substance comprising a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for the autoimmune disease. A method for preventing, delaying the onset of or treating an autoimmune disease in a patient comprising selecting a patient who is susceptible to developing the autoimmune disease, who is developing the autoimmune disease or who has the autoimmune disease and administering to the patient one or more than one dose of a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for an autoimmune disease, or comprising a polynucleotide sequence encoding the adenoviral protein E3-GP19k, or comprising a polynucleotide sequence encoding ΔBCL-2.

SUBSTANCES FOR PREVENTING AND TREATING AUTOIMMUNE DISEASES

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with United States Government support under Cooperative Agreement Number DAMD-17-97-2-7016 with the National Medical Technology Testbed, Inc., United States Department of the Army. The United States Government has certain rights in this invention.

BACKGROUND

Autoimmune diseases cause significant human morbidity and mortality. These diseases include approximately 80 diseases, such as rheumatoid arthritis, systemic lupus and multiple sclerosis, and affect approximately 5% of the population of the United States. One autoimmune disease, type 1 diabetes, is the most frequent chronic disease in children, and has a steadily increasing worldwide incidence.

Generally, the onset of type 1 diabetes begins with the display by antigen presenting cells (APCs) of autoantigens synthesized by pancreatic beta cells. This display results in the immune system destruction of pancreatic beta cells mediated mostly by T helper 1 (Th1) and cytotoxic T lymphocytes and, thereby, to the loss of insulin production.

Many prophylactic and therapeutic approaches for type 1 diabetes attempt to prevent the destruction of beta cells by inducing the immune system to delete, inactivate or suppress pathogenic self-reactive lymphocytes, such as by administering vaccines that solely deliver autoantigen, or by administering substances are direct effectors of the immune system, such as cytokines. However, currently available DNA-based vaccines are not completely efficient in preventing the disease, and the use of some of these vaccines are associated with inducing or enhancing autoimmunity rather than preventing the disease. Additionally, the use of cytokines is associated with significant morbidity.

Therefore, there is a need for a new method for preventing, delaying the onset of, or

treating autoimmune diseases using vaccines that are not associated with these disadvantages. Further, there is a need for a new method for preventing, delaying the onset of, or treating type 1 diabetes using vaccines that are not associated with these disadvantages.

SUMMARY

According to one embodiment of the present invention, there is provided a substance for preventing, delaying the onset of or treating one or more than one autoimmune disease. The substance comprises a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for the autoimmune disease.

According to another embodiment of the present invention, there is provided a use of a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for an autoimmune disease for the manufacture of a medicament for preventing, delaying the onset of or treating the one or more than one autoimmune disease.

According to another embodiment of the present invention, there is provided a use of a polynucleotide construct comprising a polynucleotide sequence encoding the adenoviral protein E3-GP19k for the manufacture of a medicament for preventing, delaying the onset of or treating one or more than one autoimmune disease.

According to another embodiment of the present invention, there is provided a use of a polynucleotide construct comprising a polynucleotide sequence encoding ΔBCL-2 for the manufacture of a medicament for preventing, delaying the onset of or treating one or more than one autoimmune disease.

In one embodiment, the medicament is manufactured in dosage units of between about 0.5 mg to about 5 mg. In another embodiment, the medicament is manufactured in dosage units of between about 1 mg to about 4 mg. In another embodiment, the medicament is manufactured in dosage units of between about 2.5 mg to about 3 mg. In another embodiment, the medicament is manufactured in a form suitable for intramuscular administration. In another embodiment, the medicament is manufactured in a form suitable for intravenous administration.

According to another embodiment of the present invention, there is provided a method for preventing, delaying the onset of or treating an autoimmune disease in a patient. The method comprises selecting a patient who is susceptible to developing the autoimmune

disease, who is developing the autoimmune disease or who has the autoimmune disease;

and administering to the patient one or more than one dose of a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for the autoimmune disease, or a polynucleotide construct comprising a polynucleotide sequence encoding the adenoviral protein E3-GP19k, or a polynucleotide construct comprising a polynucleotide sequence encoding ΔBCL-2, or a combination of the preceding polynucleotide constructs.

In one embodiment, the autoimmune disease is type I diabetes. In another embodiment, selecting the patient comprises identifying in the patient the presence of anti-insulin or anti-GAD autoantibodies or both anti-insulin and anti-GAD autoantibodies. In another embodiment, selecting the patient comprises identifying in the patient the presence of increasing hyperglycemia. In another embodiment, selecting the patient comprises identifying in the patient the presence of glycosuria. In another embodiment, selecting the patient comprises identifying in the patient the presence of a genetic predisposition to the autoimmune disease.

In another embodiment, the one or more than one dose is a plurality of doses. In another embodiment, administering to the patient one or more than one dose comprises injecting the patient intramuscularly with the one or more than one dose. In another embodiment, the method further comprises, after administering, monitoring the patient for the development the autoimmune disease.

In accordance with one aspect of the invention, there is provided the use of a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for an autoimmune disease, or a polynucleotide construct comprising a polynucleotide sequence encoding the adenoviral protein E3-GP19k, or a polynucleotide construct comprising a polynucleotide sequence encoding ΔBCL-2, or a combination of the preceding polynucleotide constructs, or a pharmaceutical composition containing said construct or constructs, for the manufacture of a medicament for the curative or prophylactic treatment of the autoimmune disease in an animal.

In accordance with another aspect of the invention, there is provided a pharmaceutical composition for the curative or prophylactic treatment of an autoimmune disease comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for the autoimmune disease, or a polynucleotide construct comprising a polynucleotide sequence encoding the adenoviral protein E3-GP19k, or a polynucleotide construct comprising a polynucleotide sequence encoding ΔBCL-2, or a combination of the preceding polynucleotide constructs together with a pharmaceutically acceptable diluent or carrier.

In accordance with another aspect of the invention, there is provided a process for the preparation of a pharmaceutical composition for the curative or prophylactic treatment of an autoimmune disease in an animal comprising by formulating a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for the autoimmune disease, or a polynucleotide construct comprising a polynucleotide sequence encoding the adenoviral protein E3-GP19k, or a polynucleotide construct comprising a polynucleotide sequence encoding ΔBCL-2, or a combination of the preceding polynucleotide constructs with a pharmaceutically acceptable diluent or carrier.

The use of a polynucleotide construct comprising a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for the autoimmune disease, or a polynucleotide construct comprising a polynucleotide sequence encoding the adenoviral protein E3-GP19k, or a polynucleotide construct comprising a polynucleotide sequence encoding ΔBCL-2, or a combination of the preceding polynucleotide constructs, or a pharmaceutical composition containing the construct or constructs, for the curative or

prophylactic treatment of an autoimmune disease in an animal.

In accordance with another aspect of the invention, there is provided a commercial package for use in the curative or prophylactic treatment of autoimmune disease in an animal containing, as active pharmaceutical ingredient, a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for the autoimmune disease, or a polynucleotide construct comprising a polynucleotide sequence encoding the adenoviral protein E3-GP19k, or a polynucleotide construct comprising a polynucleotide sequence encoding ΔBCL-2, or a combination of the preceding polynucleotide constructs, together with instructions for its use.

The medicament, polynucleotide construct, or pharmaceutical composition may be adapted for intramuscular treatment, and/or adapted for a plurality of treatments.

The autoimmune disease may be type I diabetes. The animal may be man.

FIGURES

These and other features, aspects and advantages of the present invention will become better understood with regard to the following description, appended claims, and accompanying figures where:

Figure 1 are schematic depictions of three substances according to the present invention; and

Figure 2 are schematic depictions of the fifteen plasmids that were tested for their efficiency in preventing, delaying the onset of or treating an autoimmune disease in accordance with a method of the present invention.

DESCRIPTION

According to one embodiment of the present invention, there are provided substances for preventing, delaying the onset of or treating one or more than one autoimmune disease.

According to another embodiment of the present invention, there is provided a method of preventing, delaying the onset of or treating one or more than one autoimmune disease. In one embodiment, the autoimmune disease is type 1 diabetes. In a preferred embodiment, the method comprising using a substance according to the present invention is a vaccine. The substances and method of the present invention do not use solely the delivery of autoantigen, and do not use molecules that are direct effectors of the immune system as in prior methods. Instead, the present invention uses a vaccine to prevent apoptosis of one or more than one type of cell capable of the suppressing the autoimmune disease. Because these one or more than one type of cell capable of suppressing the autoimmune disease are still be subject to physiological and immune regulation, the risk of inducing or enhancing autoimmunity is greatly reduced by the present method as compared to some prior art methods. Further, because the present invention does not involve administering substances that are direct effectors of the immune system, such as cytokines, the present invention does not pose the risk side effects associated with such direct effectors of the immune system. Further advantageously, a genetic vaccine comprising primarily plasmid DNA can be produced in large quantities at relatively low cost and does not require a "cold chain" for storage. Therefore, the substances and methods according to the present invention are both economical and practical for use to prevent, delay the onset of or treat an autoimmune disease. Further, a genetic vaccine according to the present invention modifies the genetic material of an organism directly which means that native epitopes will be processed by the organism's immune system unlike protein-based vaccines. The substances and method of the present invention will now be disclosed in detail.

As used in this disclosure, the term "autoimmune disease" comprises both diseases due in part or in total to destruction of normal cells or tissues by the organism's own immune system, and also comprises destruction of cells or tissues that were transplanted into the organism to take the place of defective or absent cells or tissues, such as islet cell transplants, or partial or whole organ transplants, by the organism's own immune system.

As used in this disclosure, the term "comprise" and variations of the term, such as "comprising" and "comprises," are not intended to exclude other additives, components, integers or steps.

In one embodiment, the present invention includes three substances that can be used either individually, sequentially or simultaneously to prevent, delay the onset of or treat one

or more than one autoimmune disease. One of the three substances is a DNA construct comprising a polynucleotide sequence, SEQ ID NO:1, encoding the pro-apoptotic protein BAX, and encoding one or more than one autoantigen for the autoimmune disease. Another of the three substances is a DNA construct comprising a polynucleotide sequence, SEQ ID NO:2, encoding the adenoviral protein E3-GP19k, which prevents presentation of an antigen on MHC-I molecules in the endoplasmic reticulum. Another of the three substances is a DNA construct comprising a polynucleotide sequence, SEQ ID NO:3, encoding a truncated form of BCL-2 designated ΔBCL-2 in this disclosure.

As will be understood by those with skill in the art with reference to this disclosure, though specific sequences are given for the polynucleotide sequences as disclosed in this disclosure, such as the polynucleotide sequences encoding the pro-apoptotic protein BAX, the adenoviral protein E3-GP19k and ΔBCL-2, the present invention includes any other sequence that does not cause a change in the translated amino acid sequence, as well as any sequence that does cause a change in the translated amino acid sequence but where the change does not substantially affect the function of the translated amino acid sequence so as to make it unsuitable for the uses contemplated in this disclosure.

Referring now to Figure 1, there are shown schematic depictions of three substances according to the present invention. As can be seen, each substance comprises a plasmid DNA construct. Substance A comprises a plasmid construct comprising a polynucleotide encoding an autoantigen for the autoimmune disease, such as secreted glutamic acid decarboxylase that is an autoantigen for type 1 diabetes, followed by a polynucleotide, SEQ ID NO:1, encoding BAX. Substance B comprises a plasmid construct comprising a polynucleotide, SEQ ID NO:2, encoding E3-GP19k without a polynucleotide encoding an autoantigen for the autoimmune disease. Substance C comprises a plasmid construct comprising a polynucleotide, SEQ ID NO:3, encoding a truncated form of the anti-apoptotic protein BCL-2 without a polynucleotide encoding an autoantigen for the autoimmune disease. As used in the Figures, "CMV" represents the cytomegalovirus promoter element, "pA" represents a polyadenylation site, and "IRES" represents an internal ribosome binding site from the EMCV virus, SEQ ID NO:4.

In order to demonstrate the advantages of the present invention, fifteen plasmids were constructed and used as vaccines. Each construct was cloned into the vector pND2. Referring now to Figure 2, there are shown schematic depictions of the fifteen plasmids that

were tested for their efficiency in preventing, delaying the onset of or treating an autoimmune disease. As can be seen, each plasmid was under the plasmid transcriptional control of the same promoter (CMVp) to ensure expression of both open reading frames in each transfected cells. During construction of these plasmids containing the cDNA encoding BCL-2, it was found that plasmid deletions occurred due to the large size of the cDNA. Therefore, a truncated version of *bcl-2* designated Δ*bcl-2* was used to construct the plasmids. As shown in Figure 2, the plasmids comprised cDNA encoding cytoplasmic GAD, SEQ ID NO:5, (plasmid 1); secreted GAD (SGAD), SEQ ID NO:6, (plasmid 2); a control secreted luciferase, SEQ ID NO:7, (plasmid 3); truncated human anti-apoptotic protein BCL-2 (ΔBCL-2), SEQ ID NO:3, (plasmid 4); anti-apoptotic protein BAX, SEQ ID NO:1, (plasmid 5); E3-GP19k, SEQ ID NO:2, (plasmid 6); ΔBCL-2, SEQ ID NO:3, in combination with cytoplasmic GAD, SEQ ID NO:5, secreted GAD, SEQ ID NO:62, and secreted luciferase, SEQ ID NO:7, (plasmids 7-9, respectively), BAX, SEQ ID NO:1, in combination with cytoplasmic GAD, SEQ ID NO:5, secreted GAD, SEQ ID NO:6, and secreted luciferase, SEQ ID NO:7, (plasmids 10-12, respectively); and E3-GP19k, SEQ ID NO:2, in combination with cytoplasmic GAD, SEQ ID NO:5, secreted GAD, SEQ ID NO:6, and secreted luciferase, SEQ ID NO:7, (plasmids 13-15, respectively).

All plasmids were generated, the open reading frame amplified using PCR, and the amplification products were inspected after DNA sequencing and found to be without mutations. Each construct was then used to transfect simian COS-7 cells transiently for immunoblot analysis of cell lysates, which confirmed that a gene product of the correct size was encoded (data not shown).

Next, the effects of the 15 plasmids on non-obese diabetic (NOD) mice were determined as follows. First, plasmid DNA was isolated using Qiagen Endorfee* kits (Qiagen Inc., Chatsworth, CA, US), and 300 ug of each of the 15 plasmid DNAs was injected intramuscularly into groups of fifteen 4-5-week-old female NOD mice. The 300 ug dose was selected as a dose relevant to the human clinical setting based on organism weight. The onset of diabetes was monitored until the age of 35 weeks, using urine and blood glucose analysis. The mice were considered diabetic after testing positive for high levels of glycosuria, with blood glucose levels greater than 300 mg/dl on two consecutive days.

The results of these experiments demonstrated the following. The percentage of diabetic animals at 35 weeks of age ranged from 73-93% for mice vaccinated with plasmids

* Trade-mark

1-3; 60-67% for mice vaccinated with plasmids 4 or 7-9; 47-85% for mice vaccinated with plasmids 5 and 10-12; and 53-73% for mice vaccinated with plasmids 6 and 13-15. Control animals (those not vaccinated) had an incidence of diabetes of about 93%. Therefore, administration of 300 ug of plasmid vector alone or of 300 ug of plasmid vector encoding antigens alone, plasmids 1-3, did not result in significant diabetes suppression. Mice vaccinated with plasmids 6-9 and 11 showed statistically significant suppression of diabetes when compared to untreated mice ($P<0.05$ for plasmid 7, and $P<0.02$ for plasmid 9). In addition, mice receiving pND2-E3-GP19k, plasmid 6 or pND2-SGAD55-BAX, plasmid 11 showed a significantly decreased incidence of diabetes at 35 weeks when compared to mice receiving plasmid pND2-GAD65, plasmid 1or pND2-GAD65-BAX, plasmid 10 ($P<0.04$), and mice receiving pND2-GAD65-ΔBCL2, plasmid 7 or pND2-SGAD55-ΔBCL2, plasmid 8 showed significantly decreased diabetes when compared to mice receiving pND2-GAD65, plasmid 1 ($P<0.05$). Suppression of diabetes was associated with decreased islet inflammation (data not shown). These results will be disclosed now in greater detail.

Mice that were vaccinated with plasmids comprising Δ*bcl-2*, plasmids 4 and 7-9, showed a 4-5 weeks delay in diabetes onset regardless of the co-expressed antigen, and a decrease in the incidence of diabetes at 35 weeks of age (60-67% compared to about 93% for the unvaccinated control mice) regardless of the co-expressed antigen. Therefore, co-expression of GAD autoantigen did not suppress the effect.

Mice that were vaccinated with plasmids comprising *bax*, plasmids 5 and 10-12, did not show diabetes suppression, with the exception of *sgad55-bax*, plasmid 11. While mice vaccinated with plasmid 11 started to develop diabetes at a time similar to other mice vaccinated with a plasmid comprising only *bax*, plasmid 5, the incidence of diabetes in mice vaccinated with plasmid 11 at 35 weeks of age was only 47% compared with a 93% incidence for the unvaccinated control mice ($p<0.05$).

Mice that were vaccinated with plasmids comprising *E3-gp19k*, plasmids 6 and 13-15 showed wide differences in diabetes onset, depending on the antigen that was co-expressed. Mice that were vaccinated with the plasmid comprising *E3-gp19k* without autoantigen, plasmid 6 started to develop diabetes with a 4-5 week delay, and showed decreased diabetes at 35 weeks of age (53% vs 93% for the unvaccinated control mice for control) ($p<0.05$). Mice that were vaccinated with the plasmids comprising *E3-gp19k* with autoantigen, plasmids 13-15, suppressed the effect, both with respect to the delay in the onset of diabetes and with

respect to the incidence of diabetic animals at 35 weeks.

Next, immune responses were characterized using a GAD-specific ELISpot* assay and ELISA of serum anti-GAD IgG isotypes to determine whether diabetes suppression by the administration of the substances of the present invention was associated with suppression of inflammatory Th1-like activity, and up-regulation of anti-inflammatory Th2 like response.

The ELISpot* assay was conducted as follows. Splenocytes were isolated from the mice at time of diabetes onset, or at the end of the observation period for non-diabetic animals. The cells were then stimulated with recombinant GAD protein, and the number of cells secreting IFN-gamma (for Th1-like activity), and IL-4 (for Th2-like activity) were counted, following a standard manufacturer's protocol. The number of cells secreting the cytokines in the absence of GAD stimulation was then subtracted, and results analyzed. For IFN-gamma the data clearly indicated that suppression of diabetes by plasmid 6, encoding E3-GP19k alone, or by plasmids 4 and 7-9, encoding ΔBCL-2 alone or together with an antigen, were associated with a suppression of GAD-specific activity. Therefore, E3-19k and ΔBCL-2 could induce an immune response that was able to suppress autoreactivity against beta cells. Surprisingly, the SGAD55-BAX combination did not appear to significantly suppress Th1-like activity. Further, SGAD55 alone, which did not suppress diabetes, did suppress GAD-specific Th1-like response.

With respect to IL-4, the data indicated an increase in GAD-specific activity for mice that received plasmid 6 encoding E3-GP19k alone (diabetes suppression), plasmid 13 encoding SGAD55 and E3-19k (no diabetes suppression), and plasmid 8 SGAD55 and ΔBCL-2(diabetes suppression). Thus, increased Th2-like activity was not always associated with decreased Th1-like activity or disease suppression.

The ELISA was conducted as follows. Animal sera were used for ELISA of anti-GAD IgG2a,b and IgG1 isotypes, which indicate a Th1-like and Th2-like activity, respectively. ELISA of anti-GAD IgG2a,b indicated that three of the plasmid DNAs coding for ΔBCL-2, plasmids 4, 8 and 9, showed a significant reduction in Th1-like activity, when compared to plasmid 5 coding for BAX, but not with the unvaccinated control mice. ELISA of anti-GAD IgG1 indicated that all plasmid DNAs encoding BAX, plasmids 5 and 10-12, resulted in decreased Th2-like activity.

These data taken together indicate that, first, *bax*, a plasmid cDNA coding for a pro-apoptotic protein, can be used as a molecular adjuvant for genetic vaccines to prevent

* Trade-mark

autoimmune disease, such as a vaccine comprising a polynucleotide encoding a secreted form of an autoantigen. Second, a plasmid cDNA encoding E3-GP19k or encoding a truncated BCL-2 alone could suppress autoimmune disease, though a plasmid cDNA encoding E3-GP19k or encoding a truncated BCL-2 combined with an autoantigen was less effective.

In one embodiment of the present invention, there is provided a method of preventing, delaying the onset of or treating an autoimmune disease. The method comprises, first, selecting a patient who is susceptible to developing the autoimmune disease, who is developing the autoimmune disease or who has the autoimmune disease. The selection can be made using standard methods as will be understood by those with skill in the art with reference to this disclosure. For example, if the autoimmune disease is diabetes, the selection can be made by identifying in the patient the presence of anti-insulin or anti-GAD autoantibodies or both anti-insulin and/or anti-GAD autoantibodies, the presence of increasing hyperglycemia, the presence of glycosuria, the presence of a genetic predisposition to diabetes or more than one of these.

Next, the patient is administered one or more than one dose of a plasmid construct according to the present invention. That is, a plasmid construct comprising a polynucleotide encoding an autoantigen for the autoimmune disease and encoding BAX, or a plasmid construct comprising a polynucleotide encoding E3-GP19k but without a polynucleotide encoding an autoantigen for the autoimmune disease, or a plasmid construct comprising a polynucleotide encoding a truncated form of the anti-apoptotic protein BCL-2 but without a polynucleotide encoding an autoantigen for the autoimmune disease. In a preferred embodiment, the organism is administered two plasmid constructs according to the present invention. In a particularly preferred embodiment, the organism is administered all three plasmid constructs according to the present invention.

In a preferred embodiment, the plasmid construct is administered in a plurality of doses. In another preferred embodiment, the dose is between about 0.001 mg/Kg and about 10 mg/Kg. In another preferred embodiment, the dose is between about 0.01 mg/Kg and about 1 mg/Kg. In another preferred embodiment, the dose is about 0.05 mg/Kg. In a preferred embodiment, a suitable dose for a human adult is between about 0.5 mg and 5 mg. In a preferred embodiment, a suitable dose for a human adult is between about 1 mg and 4 mg. In a preferred embodiment, a suitable dose for a human adult is between about 2.5 mg and 3 mg. In another preferred embodiment, the dose is administered weekly between about

2 and about 10 times. In a particularly preferred embodiment, the dose is administered weekly 4 times. In another particularly preferred embodiment, the dose is administered only once.

Administration can be by a suitable route. In a preferred embodiment, the route is intramuscular or intravenous.

Additionally, the method can comprise, after administering, monitoring the patient for the development of the autoimmune disease.

EXAMPLE I

PREVENTION OF DIABETES

According to the present invention, the onset of diabetes in a patient is delayed or prevented, for example, as follows. First, the patient is selected based on the presence of circulating anti-insulin and anti-GAD autoantibodies. Next, the patient is injected intramuscularly with 0.05 mg/Kg of a plasmid construct comprising a polynucleotide sequence, SEQ ID NO:1, encoding the pro-apoptotic protein BAX and encoding SGAD, SEQ ID NO:6, or comprising a polynucleotide sequence, SEQ ID NO:2, encoding the adenoviral protein E3-GP19k, or comprising a polynucleotide sequence, SEQ ID NO:3, encoding ΔBCL-2. The injection is repeated weekly for 3 weeks while the level of circulating anti-insulin and anti-GAD autoantibodies is monitored. The treatment is ended when the level of circulating anti-insulin and anti-GAD autoantibodies has returned to normal.

Although the present invention has been discussed in considerable detail with reference to certain preferred embodiments, other embodiments are possible. Therefore, the scope of the appended claims should not be limited to the description of preferred embodiments contained in this disclosure.

SEQUENCE LISTING

<110> Loma Linda University
ESCHER, Alan P.
LI, Fengchun

<120> Substances for Preventing and Treating Autoimmune Diseases

<130> 14102-1PCT

<140> to be assigned
<141> 2003-08-06

<150> US 60/401,652
<151> 2002-08-06

<160> 7

<170> PatentIn version 3.2

<210> 1
<211> 579
<212> DNA
<213> Homo sapiens

<400> 1
atggacgggt ccggggagca gcccagaggc gggggggccca ccagctctga gcagatcatg 60

aagacagggg ccctttgct tcagggtttc atccaggatc gagcagggcg aatgggggggg 120

gaggcacccg agctggccct ggacccggtg cctcaggatg cgtccaccaa gaagctgagc 180

gagtgtctca agcgcatcgg ggacgaactg gacagtaaca tggagctgca gaggatgatt 240

gccgccgtgg acacagactc cccccgagag gtctttttcc gagtggcagc tgacatgttt 300

tctgacggca acttcaactg gggccgggtt gtcgcccttt tctactttgc cagcaaactg 360

gtgctcaagg ccctgtgcac caaggtgccg gaactgatca gaaccatcat gggctggaca 420

ttggacttcc tccgggagcg gctgttgggc tggatccaag accagggtgg ttgggacggc 480

ctcctctcct actttgggac gcccacgtgg cagaccgtga ccatctttgt ggcgggagtg 540

ctcaccgcct cgctcaccat ctggaagaag atgggctga 579

<210> 2
<211> 492
<212> DNA
<213> Human adenovirus type 2

<400> 2
atgaggtaca tgattttagg cttgctcgcc cttgcggcag tctgcagcgc tgccaaaaag 60

gttgagttta aggaaccagc ttgcaatgtt acatttaaat cagaagctaa tgaatgcact 120

actcttataa aatgcaccac agaacatgaa aagcttatta ttcgccacaa agacaaaatt 180

ggcaagtatg ctgtatatgc tatttggcag ccaggtgaca ctaacgacta taatgtcaca 240

gtcttccaag gtgaaaatcg taaaactttt atgtataaat ttccatttta tgaaatgtgc 300

gatattacca tgtacatgag caaacagtac aagttgtggc ccccacaaaa gtgtttagag 360

aacactggca ccttttgttc caccgctctg cttattacag cgcttgcttt ggtatgtacc 420

ttactttatc tcaaatacaa aagcagacgc agttttattg atgaaaagaa aatgccttga 480

ttttccgctt gc 492

<210> 3
<211> 599
<212> DNA
<213> Homo sapiens

<400> 3
atggcgcacg ctgggagaag tggttacgat aaccgggaga tagtgatgaa gtacatccat 60

tataagctgt cgcagagggg ctacgagtgg gatgctaccg cggctgccgc ggggcctgcg 120

ctcagcccgg tgccacctgt ggtccacctg accctccgcc aggccggcga cgacttctcc 180

cgccgctacc gccgcgactt cgccgagatg tccagccagc tgcacctgac gcccttcacc 240

gcgcggggat gctttgccac ggtggtggag gagctcttca gggacggggt gaactggggg 300

aggattgtgg ccttctttga gttcggtggg gtcatgtgtg tggagagcgt caaccgggag 360

atgtcgcccc tggtggacaa catcgccctg tggatgactg agtacctgaa ccggcacctg 420

cacacctgga tccaggataa cggaggctgg gatgcctttg tggaactgta cggccccagc 480

atgcggcctc tgtttgattt ctcctggctg tctctgaaga ctctgctcag tttggccctg 540

gtgggagctt gcatcaccct gggtgcctat ctgggccaca agtgaagtca acatgcctg 599

<210> 4
<211> 619
<212> DNA
<213> Encephalomyocarditis virus

<400> 4
tctagataat acgactcact atagggcgaa ttccccctct ccctcccccc cccctaacgt 60

tactggccga agccgcttgg aataaggccg gtgtgcgttt gtctatatgt tattttccac 120

catattgccg tcttttggca atgtgagggc ccggaaacct ggccctgtct tcttgacgag 180

cattcctagg ggtctttccc ctctcgccaa aggaatgcaa ggtctgttga atgtcgtgaa 240

ggaagcagtt cctctggaag cttcttgaag acaaacaacg tctgtagcga ccctttgcag 300

gcagcggaac cccccacctg gcgacaggtg cctctgcggc caaaagccag gtgtataaga 360

tacacctgca aaggcggcac aaccccagtg ccacgttgtg agttggaata gttgtggaaa 420

gagtcaaatg gctctcctca agcgtattca acaaggggct gaaggatgcc cagaaggtac 480

cccattgtat gggatctgat ctggggcctc ggtgcacatg ctttacatgt gtttagtcga 540

ggttaaaaaa cgtctaggcc ccccaaccac ggggacgtgg ttttcctttg aaaaacacga 600

ttattatatt gcctctaga 619

<210> 5
<211> 1868
<212> DNA
<213> Homo sapiens

<400> 5
gagctccacc gcggtggcgg ccgctctaga ccaccatggc atctccgggc tctggctttt 60

ggtctttcgg gtcggaagat ggctctgggg attccgagaa tcccggcaca gcgcgagcct 120

ggtgccaagt ggctcagaag ttcacgggcg gcatcggaaa caaactgtgc gccctgctct 180

acggagacgc cgagaagccg gcggagagcg gcgggagcca acccccgcgg gccgccgccc 240

ggaaggccgc ctgcgcctgc gaccagaagc cctgcagctg ctccaaagtg gatgtcaact 300

acgcgtttct ccatgcaaca gacctgctgc cggcgtgtga tggagaaagg cccactttgg 360

cgtttctgca agatgttatg aacattttac ttcagtatgt ggtgaaaagt ttcgatagat 420

caaccaaagt gattgatttc cattatccta atgagcttct ccaagaatat aattgggaat 480

tggcagacca accacaaaat ttggaggaaa ttttgatgca ttgccaaaca actctaaaat 540

atgcaattaa aacagggcat cctagatact tcaatcaact ttctactggt ttggatatgg 600

ttggattagc agcagactgg ctgacatcaa cagcaaatac taacatgttc acctatgaaa 660

ttgctccagt atttgtgctt ttggaatatg tcacactaaa gaaaatgaga gaaatcattg 720

gctggccagg gggctctggc gatgggatat tttctcccgg tggcgccata tctaacatgt 780

atgccatgat gatcgcacgc tttaagatgt tcccagaagt caaggagaaa ggaatggctg 840

ctcttcccag gctcattgcc ttcacgtctg aacatagtca tttttctctc aagaagggag 900

ctgcagcctt agggattgga agagacagcg tgattctgat taaatgtgat gagagaggga 960

aaatgattcc atctgatctt gaaagaagga ttcttgaagc caaacagaaa gggtttgttc 1020

ctttcctcgt gagtgccaca gctggaacca ccgtgtacgg agcatttgac cccctcttag 1080

ctgtcgctga catttgcaaa aagtataaga tctggatgca tgtggatgca gcttggggtg 1140

ggggattact gatgtcccga aaacacaagt ggaaactgag tggcgtggag agggccaact 1200

ctgtgacgtg gaatccacac aagatgatgg gagtcccttt gcagtggtct gctctcctgg 1260

ttagagaaga gggattgatg cagaattgca accaaatgca tgcctcctac ctctttcagc 1320

aagataaaca ttatgacctg tcctatgaca ctggagacaa ggccttacag tgcggacgcc 1380

acgttgatgt ttttaaacta tggctgatgt ggagggcaaa ggggactacc gggtttgaag 1440

cgcatgttga taaatgtttg gagttggcag agtatttata caacatcata aaaaaccgag 1500

aaggatatga gatggtgttt gatgggaagc ctgaggacac aaatgtctgc ttctggtaca 1560

ttcctccaag cttgcgtact ctggaagaca atgaagagag aatgagtcgc ctctcgaagg 1620

tggctccagt gattaaagcc agaatgatgg agtatggaac cacaatggtc agctaccaac 1680

ccttgggaga caaggtcaat ttcttccgca tggtcatctc aaacccagcg gcaactcacc 1740

aagacattga cttcctgatt gaagaaatag aacgccttgg acaagattta taataacctt 1800

gctcaccaag ctgttccact tctctaggta gcgacctcga gcggccgctc gagggggggc 1860

ccggtacc 1868

<210> 6
<211> 1638
<212> DNA
<213> Artificial

<220>
<223> secreted form of human GAD

<400> 6
atgtacagga tgcaactcct gtcttgcatt gcactaagtc ttgcacttgt cacaaacagt 60

gcacctactt acgcgtttct ccatgcaaca gacctgctgc cggcgtgtga tggagaaagg 120

cccactttgg cgtttctgca agatgttatg aacattttac ttcagtatgt ggtgaaaagt 180

ttcgatagat caaccaaagt gattgatttc cattatccta atgagcttct ccaagaatat 240

aattgggaat tggcagacca accacaaaat ttggaggaaa ttttgatgca ttgccaaaca 300

actctaaaat atgcaattaa aacagggcat cctagatact tcaatcaact ttctactggt 360

ttggatatgg ttggattagc agcagactgg ctgacatcaa cagcaaatac taacatgttc 420

acctatgaaa ttgctccagt atttgtgctt ttggaatatg tcacactaaa gaaaatgaga 480

gaaatcattg gctggccagg gggctctggc gatgggatat tttctcccgg tggcgccata 540

tctaacatgt atgccatgat gatcgcacgc tttaagatgt tcccagaagt caaggagaaa 600

ggaatggctg ctcttcccag gctcattgcc ttcacgtctg aacatagtca tttttctctc 660

aagaagggag ctgcagcctt agggattgga agagacagcg tgattctgat taaatgtgat 720

gagagaggga aaatgattcc atctgatctt gaaagaagga ttcttgaagc caaacagaaa 780

gggtttgttc ctttcctcgt gagtgccaca gctggaacca ccgtgtacgg agcatttgac 840

ccctcttag ctgtcgctga catttgcaaa aagtataaga tctggatgca tgtggatgca 900

gcttggggtg ggggattact gatgtcccga aaacacaagt ggaaactgag tggcgtggag 960

agggccaact ctgtgacgtg gaatccacac aagatgatgg gagtcccttt gcagtggtct 1020

gctctcctgg ttagagaaga gggattgatg cagaattgca accaaatgca tgcctcctac 1080

ctctttcagc aagataaaca ttatgacctg tcctatgaca ctggagacaa ggccttacag 1140

tgcggacgcc acgttgatgt ttttaaacta tggctgatgt ggagggcaaa ggggactacc 1200

gggtttgaag cgcatgttga taaatgtttg gagttggcag agtatttata caacatcata 1260

aaaaaccgag aaggatatga gatggtgttt gatgggaagc ctgaggacac aaatgtctgc 1320

ttctggtaca ttcctccaag cttgcgtact ctggaagaca atgaagagag aatgagtcgc 1380

ctctcgaagg tggctccagt gattaaagcc agaatgatgg agtatggaac cacaatggtc 1440

agctaccaac ccttgggaga caaggtcaat ttcttccgca tggtcatctc aaacccagcg 1500

gcaactcacc aagacattga cttcctgatt gaagaaatag aacgccttgg acaagattta 1560

taataacctt gctcaccaag ctgttccact tctctaggta gcgacctcga gcggccgctc 1620

gagggggggc ccggtacc 1638

<210> 7
<211> 1271
<212> DNA
<213> Artificial

<220>
<223> secreted form of Renilla luciferase

<400> 7
atgtacagga tgcaactcct gtcttgcatt gcactaagtc ttgcacttgt cacaaacagt 60

gcacctactg aattcagctt aaagatgact tcgaaagttt atgatccaga acaaaggaaa 120

cggatgataa ctggtccgca gtggtgggcc agatgtaaac aaatgaatgt tcttgattca 180

tttattaatt attatgattc agaaaaaacat gcagaaaatg ctgttatttt tttacatggt 240

aacgcggcct cttcttattt atggcgacat gttgtgccac atattgagcc agtagcgcgg 300

tgtattatac cagatcttat tggtatgggc aaatcaggca aatctggtaa tggttcttat 360

aggttacttg atcattacaa atatcttact gcatggtttg aacttcttaa tttaccaaag 420

aagatcattt ttgtcggcca tgattggggt gctgctttgg catttcatta tagctatgag 480

catcaagata agatcaaagc aatagttcac gctgaaagtg tagtagatgt gattgaatca 540

tgggatgaat ggcctgatat tgaagaagat attgcgttga tcaaatctga agaaggagaa 600

aaaatggttt tggagaataa cttcttcgtg gaaaccatgt tgccatcaaa aatcatgaga 660

aagttagaac cagaagaatt tgcagcatat cttgaaccat tcaaagagaa aggtgaagtt 720

cgtcgtccaa cattatcatg gcctcgtgaa atcccgttag taaaaggtgg taaacctgac 780

gttgtacaaa ttgttaggaa ttataatgct tatctacgtg caagtgatga tttaccaaaa 840

atgtttattg aatcggatcc aggattcttt tccaatgcta ttgttgaagg cgccaagaag 900

tttcctaata ctgaatttgt caaagtaaaa ggtcttcatt tttcgcaaga agatgcacct 960

gatgaaatgg gaaaatatat caaatcgttc gttgagcgag ttctcaaaaa tgaacaataa 1020

ttactttggt tttttattta catttttccc gggtttaata atataaatgt cattttcaac 1080

aattttattt taactgaata tttcacaggg aacattcata tatgttgatt aatttagctc 1140

gaactttact ctgtcatatc attttggaat attacctctt tcaatgaaac tttataaaca 1200

gtggttcaat taattaatat atattataat tacatttgtt atgtaataaa ctcggtttta 1260

ttataaaaaa a 1271

WHAT IS CLAIMED IS:

1. A polynucleotide for preventing, delaying the onset of or treating type I diabetes in an animal, the polynucleotide comprising a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding a secreted glutamic acid decarboxylase (sGAD).

2. Use of a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding a secreted glutamic acid decarboxylase (sGAD) for the manufacture of a medicament for preventing, delaying the onset of, or treating type I diabetes in an animal.

3. Use of a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding a secreted glutamic acid decarboxylase (sGAD) for preventing, delaying the onset of, or treating type I diabetes in an animal.

4. Use according to claim 2 or 3, wherein the polynucleotide construct is for use in dosage units of between about 0.5 mg to about 5 mg.

5. Use according to claim 2 or 3, wherein the polynucleotide construct is for use in dosage units of between about 1 mg to about 4 mg.

6. Use according to claim 2 or 3, wherein the polynucleotide construct is for use in dosage units of between about 2.5 mg to about 3 mg.

7. Use according to claim 2 or 3, wherein the polynucleotide construct is for intramuscular administration.

8. Use according to claim 2 or 3, wherein the polynucleotide construct is for intravenous administration.

9. A pharmaceutical composition for the curative or prophylactic treatment of type I diabetes in an animal comprising a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding a secreted glutamic acid decarboxylase (sGAD), together with a pharmaceutically acceptable diluent or carrier.

10. A process for the preparation of a pharmaceutical composition for the curative or prophylactic treatment of type I diabetes in an animal comprising formulating a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding a secreted glutamic acid decarboxylase (sGAD) with a pharmaceutically acceptable diluent or carrier.

11. A commercial package for use in the curative or prophylactic treatment of type I diabetes in an animal, containing as active pharmaceutical ingredient a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding a secreted glutamic acid

decarboxylase (sGAD), together with instructions for use of the commercial package.

12. The pharmaceutical composition of claim 9 wherein the polynucleotide construct is for intramuscular administration.

13. The process of claim 10, wherein the pharmaceutical composition is for intramuscular administration.

14. The commercial package of claim 11 wherein the polynucleotide sequence is for intramuscular administration.

15. The use of any one of claims 2 to 8, wherein the polynucleotide construct is for use in a plurality of doses.

16. The pharmaceutical composition of claim 9 or 12, wherein the polynucleotide construct is for use in a plurality of doses.

17. The process of claim 10 or 13, wherein the polynucleotide construct is for use in a plurality of doses.

18. The commercial package of claim 11 or 14 wherein the polynucleotide sequence is for use in a plurality of doses.

19. The polynucleotide of claim 1 wherein the animal is man.

20. The use according to any one of claims 2 to 8, or 15, wherein the animal is man.

21. The pharmaceutical composition of claim 9, 12 or 16 wherein the animal is man.

22. The process of claim 10, 13 or 17 wherein the animal is man.

23. The commercial package of claim 11, 14 or 18 wherein the animal is man.

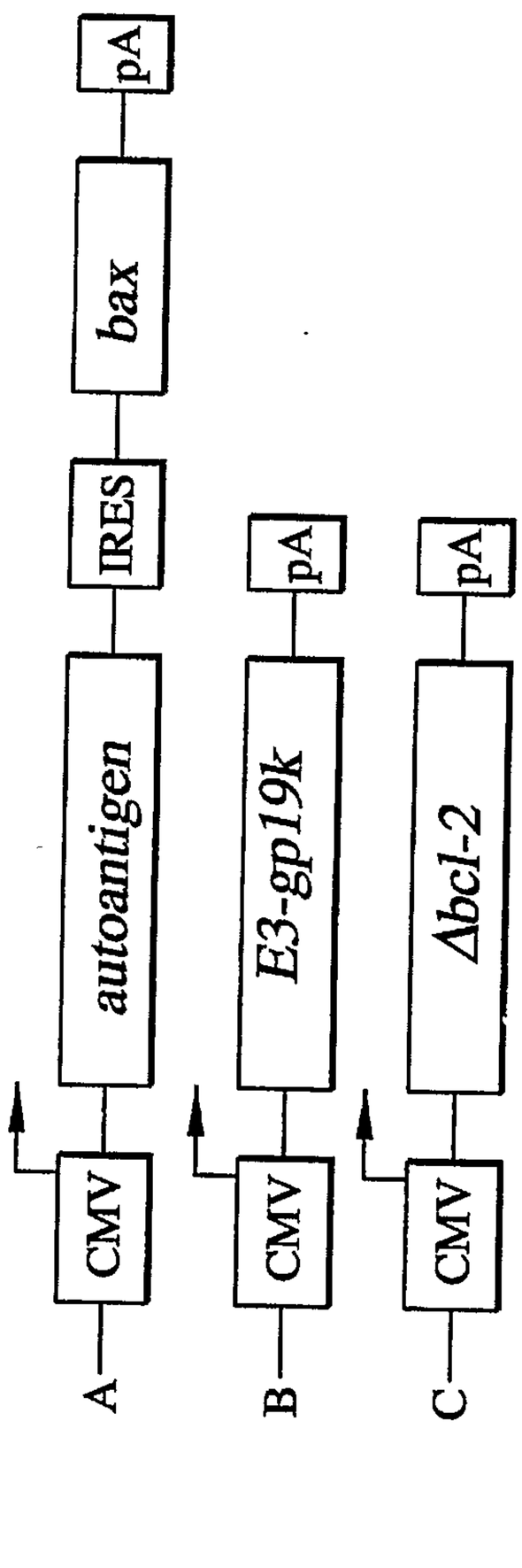

FIG. 1

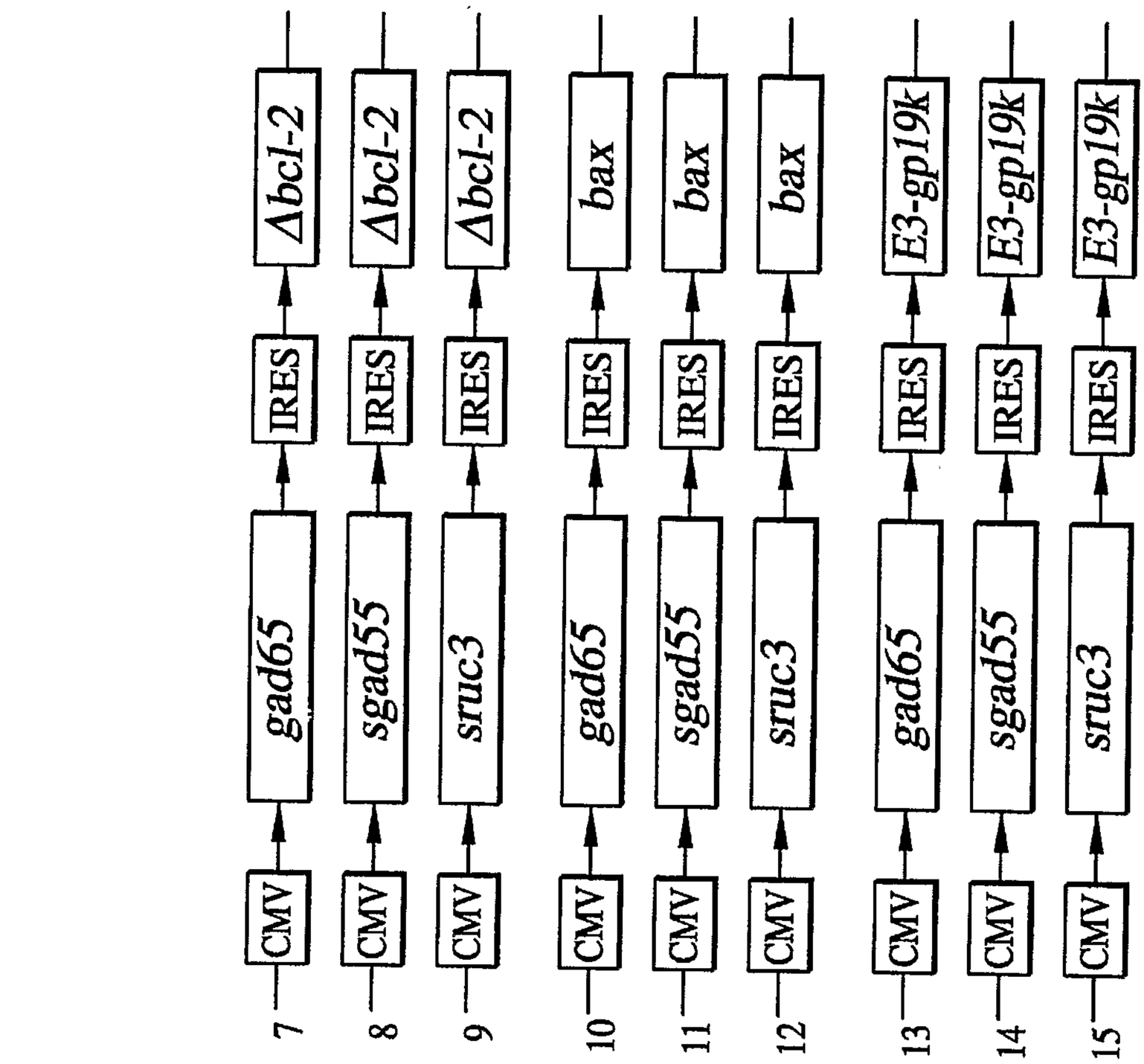
CMV
gad65
IRES
Δbcl-2
1
CMV
sgad55
IRES
Δbcl-2
2
CMV
sruc3
IRES
Δbcl-2
3
CMV
gad65
IRES
bax
4
CMV
sgad55
IRES
bax
5
CMV
sruc3
IRES
bax
6
CMV
gad65
IRES
E3-gp19k
7
CMV
sgad55
IRES
E3-gp19k
8
CMV
sruc3
IRES
E3-gp19k
9
CMV
gad65
Δbcl-2
10
CMV
sgad55
bax
11
CMV
sruc3
E3-gp19k
12
CMV
gad65
E3-gp19k
13
CMV
sgad55
E3-gp19k
14
CMV
sruc3
E3-gp19k
15

FIG.2