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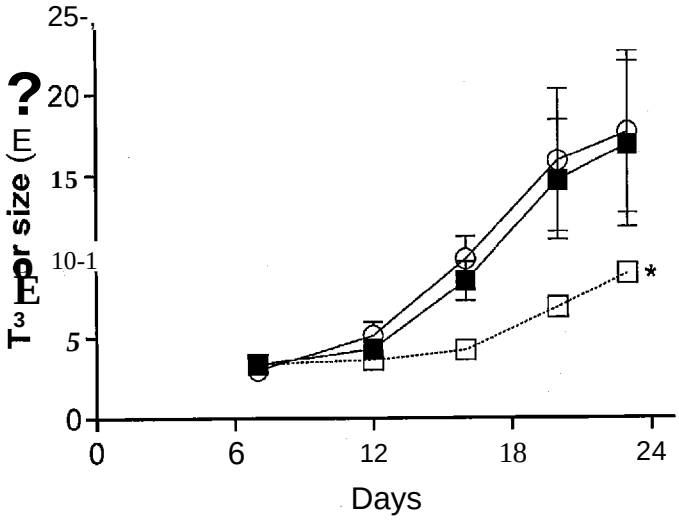
(54) Title: COMPOSITIONS FOR MODULATING AN XBPl PATHWAY IN A KERATINOCYTE AND METHODS OF USE

(57) Abstract: Provided herein are compositions and methods for increasing an immune response in a subject, immunizing a subject and/or treating a disease in a subject that relate to keratinocytes. It is a surprising finding of the present invention that modulation of an XBPl pathway creates a keratinocyte that is itself an immune modulator, or adjuvant. In some embodiments, the concentration of antigen is increased in the vicinity of the keratinocyte, further increasing the immune response by effector cells within that vicinity.

FIGURE 7



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COMPOSITIONS FOR MODULATING AN XBPl PATHWAY IN A KERATINOCYTE AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of U.S. Provisional Application No. 62/184,287, filed June 25, 2015, which is hereby incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

1) FIELD OF THE INVENTION

The field of the invention is immunology.

2) DESCRIPTION OF RELATED ART

The skin can be a uniquely immunogenic target for immunization, as it contains an extensive array of immunologically responsive cell types that contribute to both innate and adaptive immunity. Strategic engineering of the skin to create a pro-immunogenic microenvironment could lead to more effective preventive and therapeutic vaccines against dreaded diseases such as cancer and AIDS.

The transcription factor x-box binding protein 1 (spliced) XBPl, which is an endoplasmic reticulum (ER)-stress associated factor regulating ER structure and function, can promote the production and secretion of proteins, and regulate cell differentiation and survival in certain cells. However, the regulatory networks affected by XBPl are not well understood.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure l(A-D) contains graphs showing XBPl enhances the production of secreted vaccine antigen and pro-inflammatory cytokines by keratinocytes. OVA (Fig. 1A), IL-la (Fig. IB), IFN-β (Fig. 1C), CCL2 (Fig. ID).

Figure 2(A-F) contains graphs showing that XBPl promotes lymphocyte and CDllc $^{+}$ cell infiltration into skin. Fig. 2A-B, untreated. Fig. 2C-D, Control vector + OVA. Fig. 2E-F, XBPl + OVA.

Figure 3(A-F) contains graphs showing that XBPl *in vivo* increases the expression of pro-inflammatory cytokines and chemokines and co-delivered vaccine antigen transgene. XBPl (Fig. 3A), OVA (Fig. 3B), CCL2 (Fig. 3C), TNF-a (Fig. 3D), IL-la (Fig. 3E), IL-Iβ (Fig. 3F).

Figure 4(A-E) contains graphs showing that XBP1 overexpression enables induction of durable systemic antigen- specific IFN-γ- and Granzyme B-expressing $CD8^+$ T cell immunity. Fig. 4B showing untreated, restimulation with OVA-specific SIINFEKL. Fig.4C showing control vector + OVA, restimulation with OVA-specific SIINFEKL. Fig. 4D showing XBP1 + OVA, restimulation with OVA-specific SIINFEKL, Fig. 4E showing XBP1 +OVA, restimulation with β-gal-specific DAPIYTNV.

Figure 5(A-F) contains graphs showing that XBP1 promotes the accumulation of memory [central ($CD44^+CD62L^+$) and effector ($CD44^+CD62L^-$)] $CD8^+$ T cells and skin-resident $CD103^+CD8^+$ memory T cells in skin at the immunization site. Fig. 5A-B, untreated. Fig. 5C-D, Control vector + OVA. Fig. 5E-F, XBP1 + OVA.

Figure 6(A-0) contains graphs showing that overexpression of XBP1 *in situ* (Fig. 6A) triggers expression of known XBP1 responsive genes (GRP78, GFAT-1 and VEGFA; Figs. 6B, 6C, 6D), and genes associated with keratinocytes migration, proliferation and function (HIF-1α Fig. 6E), pro-inflammatory responses (IL-23a, S100A7, IL-1β, MyD88, OAS1, TNF-a; Figs. 6F, 6G, 61, 6K, 6N), the recruitment and activation of immunocytes (CCL19, CD86 and IL-15; Figs. 6H, 6L, 6M), and co-delivered vaccine antigen transgene (OVA; Fig. 60).

Figure 7 contains a graph showing that transient overexpression of XBP1 in skin drives vaccine-induced durable protective immunity. Untreated (filled squares), control vector plus OVA (open circles), XBP1 plus OVA (open squares).

DETAILED DESCRIPTION OF THE INVENTION

Provided herein are compositions and methods for increasing an immune response in a subject, immunizing a subject and/or treating a disease in a subject that relate to keratinocytes. Terms used throughout this application are to be construed with ordinary and typical meaning to those of ordinary skill in the art. However, Applicant desires that the following terms be given the particular definition as defined below.

Definitions

As used in the specification and claims, the singular form "a," "an," and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof.

The term "administering" refers to an administration that is oral, topical, intravenous, cutaneous, subcutaneous, transcutaneous, transdermal, intramuscular, intra-joint, parenteral, intra-arteriole, intradermal, intraventricular, intracranial, intraperitoneal, intralesional, intranasal, rectal,

vaginal, by inhalation or via an implanted reservoir. The term "parenteral" includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional, and intracranial injections or infusion techniques. In one embodiment, the administration is cutaneous or transdermal. It should be understood that a cutaneous administration does not require systemic delivery of the administered composition.

The terms "about" and "approximately" are defined as being "close to" as understood by one of ordinary skill in the art. In one non-limiting embodiment, the terms are defined to be within 10%. In another non-limiting embodiment, the terms are defined to be within 5%. In still another non-limiting embodiment, the terms are defined to be within 1%.

The term "antigen" refers to any composition toward which an immune response is generated. Antigens include, but are not limited to, polypeptides, oligopeptides, and polysaccharides. In one embodiment, the antigen is a polypeptide.

The terms "cell," "cell line," and "cell culture" include progeny. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Variant progeny within a population, which population has the same overexpression of XBP1 as screened for in the originally engineered cell population, are included.

A "composition" is intended to include a combination of active agent or agents (for example, an XBP1 pathway upregulating composition) and another compound or composition, inert (for example, a detectable agent or label) or active, such as an adjuvant.

As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like. "Consisting of shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

A "control" is an alternative subject or sample used in an experiment for comparison purpose. A control can be "positive" or "negative."

The term "disease" refers to an abnormal condition of a part, organ, or system of a subject resulting from various causes, such as infection, inflammation, environmental factors, or genetic defect, and characterized by an identifiable group of signs, symptoms, or both. In some embodiments, the disease is a cancer.

An "effective amount" is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages.

As used herein, "gene expression" and "protein expression" refer to the process by which polynucleotides are transcribed into mRNA and the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins, respectively. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in a eukaryotic cell. "Gene overexpression" refers to the overproduction of the mRNA transcribed from the gene, at a level that is about 2.5 times higher, about 5 times higher, or about 10 times higher than the expression level detected in a control sample. "Protein overexpression" includes the overproduction of the protein product encoded by a gene at a level that is about 2.5 times higher, about 5 times higher, or about 10 times higher than the expression level detected in a control sample.

As used herein "surface expression" refers to the process by which polypeptides are translocated to the surface of a cell such that at least a portion of the polypeptide is located at the exterior of the cell surface. "Surface overexpression" includes an increase in the amount of a particular polypeptide at the exterior surface of a cell, at a level that is about 2.5 times higher, about 5 times higher, or about 10 times higher than the surface expression level detected in a control sample.

A "gene" refers to a polynucleotide containing at least one open reading frame that is capable of encoding a particular polypeptide or protein after being transcribed and translated. Any of the polynucleotides sequences described herein may be used to identify larger fragments or full-length coding sequences of the gene with which they are associated. Methods of isolating larger fragment sequences are known to those of skill in the art.

"Homologs" are defined herein as two polynucleotides or two polypeptides that have identity or homology. Homologs include allelic variants, orthologs, and paralogs having the same relevant function (e.g., ability to upregulate the XBP1 pathway). In some embodiments, homologs have about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92, 91% or 90% homology. In other embodiments, homologs have about 80% or about 85% homology.

The term "identity" or "homology" shall be construed to mean the percentage of nucleotide bases or amino acid residues in the candidate sequence that are identical with the bases or residues of a corresponding sequence to which it is compared, after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent identity for the entire sequence, and not considering any conservative substitutions as part of the sequence identity. Neither N- nor C-terminal extensions nor insertions shall be construed as reducing identity or homology. A

polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) that has a certain percentage (for example, 80%, 85%, 90%, or 95%) of "sequence homology" to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art. In one embodiment, default parameters are used for alignment. In one embodiment a BLAST program is used with default parameters. In one embodiment, BLAST programs BLASTN and BLASTP are used with the following default parameters: Genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by=HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+GenBank CDS translations+SwissProtein+SPupdate+PIR.

The term "immunizing" refers to increasing an antigen-specific immune response in a subject.

The term "keratinocyte" refers to an epidermal cell that expresses one or more keratin polypeptides. The term "keratinocyte" includes keratinocytes at each stage of differentiation, but does not include corneocytes. In some embodiments, the keratinocyte expresses or overexpresses a keratin-5 polypeptide and a keratin-14 polypeptide. In other embodiments, the keratinocyte expresses or overexpresses a keratin-1 polypeptide and a keratin-10 polypeptide. In still other embodiments, the keratinocyte expresses or overexpresses a keratin-1 polypeptide and a keratin-16 polypeptide.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including human, domestic and farm animals, nonhuman primates, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc.

As used herein, the terms "neoplastic cells," "neoplasia," "tumor cells," "tumor," "cancer," and "cancer cells" (used interchangeably) refer to cells which exhibit relatively autonomous growth, so that they exhibit an aberrant growth phenotype characterized by a significant loss of control of cell proliferation (i.e., de-regulated cell division). Tumor cells can be malignant or benign. A metastatic cell or tissue means that the cell can invade and destroy neighboring body structures. In some embodiments, the cancer is a skin cancer such as a melanoma, a basal cell carcinoma or a squamous cell carcinoma.

A "pharmaceutical composition" is intended to include the combination of an active agent with a pharmaceutically acceptable carrier, inert or active, making the composition suitable for diagnostic or therapeutic use *in vivo* or *ex vivo*.

The term "pharmaceutically acceptable carrier" means a carrier or excipient that is useful in preparing a pharmaceutical composition that is generally safe and non-toxic, and includes a carrier that is acceptable for veterinary and/or human pharmaceutical use. As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. As used herein, the term "carrier" encompasses any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, lipid, stabilizer, or other material well known in the art for use in pharmaceutical formulations and as described further below. The pharmaceutical compositions also can include preservatives. A "pharmaceutically acceptable carrier" as used in the specification and claims includes both one and more than one such carrier.

The terms "pharmaceutically effective amount," "therapeutically effective amount," or "therapeutically effective dose" refer to the amount of a composition such as an XBP1 pathway upregulating composition, and optionally, antigen polynucleotide, that will elicit the biological or medical response of a tissue, system, animal, or human that is being sought by the researcher, veterinarian, medical doctor or other clinician. In some embodiments, a desired response is a treatment of a disease such as a bacterial infection, a viral infection or a cancer such as a skin cancer. In some instances, a desired biological or medical response is achieved following administration of multiple dosages of the composition to the subject over a period of days, weeks, or years. The terms "pharmaceutically effective amount," "therapeutically effective amount," or "therapeutically effective dose" include that amount of a composition such as a XBP1 pathway upregulating composition, and optionally, antigen polynucleotide, that, when administered, is sufficient to prevent development of, or alleviate to some extent, one or more of the symptoms of the disease being treated. The therapeutically effective amount will vary depending on the composition such as the a XBP1 pathway upregulating composition, and optionally, antigen polynucleotide, the disease and its severity, the route of administration, time of administration, rate of excretion, drug combination, judgment of the treating physician, dosage form, and the age, weight, general health, sex and/or diet of the subject to be treated. In the context of the present method, a pharmaceutically or therapeutically effective amount or dose of a XBP1 pathway upregulating composition, and optionally, antigen polynucleotide, includes an amount that is sufficient to prevent development of, suppress the growth of, or reduce the numbers of, one or more skin cancer lesions.

The terms "polynucleotide" and "oligonucleotide" are used interchangeably, and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or

analogs thereof. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. The term also refers to both double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of this invention that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form. As used herein, an "antigen polynucleotide" is a polynucleotide that encodes a corresponding antigen polypeptide.

A polynucleotide is composed of a specific sequence of four nucleotide bases: adenine (A); cytosine (C); guanine (G); thymine (T); and uracil (U) for thymine (T) when the polynucleotide is RNA. Thus, the term "polynucleotide sequence" is the alphabetical representation of a polynucleotide molecule. This alphabetical representation can be input into databases in a computer having a central processing unit and used for bioinformatics applications such as functional genomics and homology searching.

The term "polypeptide" is used in its broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogs, or peptidomimetics. The subunits may be linked by peptide bonds. In another embodiment, the subunit may be linked by other bonds, e.g. ester, ether, etc. As used herein the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics. A peptide of three or more amino acids is commonly called an oligopeptide if the peptide chain is short. If the peptide chain is long, the peptide is commonly called a polypeptide or a protein.

The terms "prevent," "preventing," "prevention," and grammatical variations thereof as used herein, refer to a method of partially or completely delaying or precluding the onset or recurrence of a disease and/or one or more of its attendant symptoms or barring a subject from acquiring or reacquiring a disease or reducing a subject's risk of acquiring or reacquiring a disease or one or more of its attendant symptoms.

The term "subject" is defined herein to include animals such as mammals, including, but not limited to, primates (e.g., humans), cows, sheep, goats, horses, dogs, cats, rabbits, rats, mice and the like. In some embodiments, the subject is a human.

"Suppress" tumor growth indicates a curtailment of growth state when compared to growth without contact with a XBP1 pathway upregulating composition and antigen polynucleotide described herein. Tumor cell growth can be assessed by any means known in the art, including, but not limited to, measuring tumor size, determining whether tumor cells are proliferating using a ^{3}H-thymidine incorporation assay, or counting tumor cells. "Suppressing" tumor cell growth means any or all of the following states: slowing, delaying, and stopping tumor growth, as well as tumor shrinkage.

The terms "treat," "treating," "treatment" and grammatical variations thereof as used herein, include partially or completely delaying, alleviating, mitigating or reducing the intensity of one or more attendant symptoms of a disease and/or alleviating, mitigating or impeding one or more causes of a disease. Treatments according to the invention may be applied preventively, prophylactically, pallatively or remedially. Prophylactic treatments are administered to a subject prior to onset *(e.g. ,* before obvious signs of disease), during early onset *(e.g. ,* upon initial signs and symptoms of disease), or after an established development of disease. Prophylactic administration can occur for several days to years prior to the manifestation of symptoms of an infection. In some instances, the terms "treat," "treating," "treatment" and grammatical variations thereof, include partially or completely reducing the size of a solid tumor or cancer lesion or reducing the number of solid tumors or cancer lesions as compared with prior to treatment of the subject or as compared with the incidence of such symptom in a general or study population.

The term "vector" means a DNA construct containing a DNA sequence which is operably linked to a suitable control sequence capable of effecting the expression of the DNA in a suitable host. Such control sequences include a promoter to effect transcription, an optional operator sequence to control such transcription, a sequence encoding suitable mRNA ribosome binding sites, and sequences which control the termination of transcription and translation. The vector may be a plasmid, a phage particle, or simply a potential genomic insert. Once transformed into a suitable host, the vector may replicate and function independently of the host genome, or may in some instances, integrate into the genome itself. A plasmid is the most commonly used form of vector, however, the invention is intended to include such other forms of vectors which serve equivalent function as and which are, or become, known in the art.

The term "XBP1 pathway upregulating composition" refers herein to any composition that when administered to a keratinocyte, increases or activates a constituent in an XBP1

regulated/responsive pathway. For example, downstream pathways known to be effected by XBP1 include those of the ER stress Gene Network including, for example, Sec24c, Sec31a, Sec23b, Sec24d, Sec61al, Copgl, Copb2, Gosr2, Golgbl, Golga3, Arfgap3, Rpn2, Spcs3, Fasn, Hspal3, Surf4, Jnk, Mfn2, Atf6, Dnajc3, Pdia6, Pdia5, Pdia4, Rpnl, Os9, Hyoul, Csdc47, Stt3a, and Nlrxl. Other downstream pathways known to be effected by XBP1 include: Protein transport pathways including, for example, those effecting GRP78; Cell metabolism pathways including, for example, those effecting GFAT-1; Pathways effecting blood vessel growth including, for example, those effecting VEGFA; Pathways effecting triglyceride biosynthesis including, for example, those effecting Agpat6, Fasn, Scd2, or Lparl; Pathways effecting keratinocytes migration, proliferation and function including, for example, those effecting HIF-Iα and IL-la; Pathways effecting innate immune signaling including, for example, those effecting MyD88, OAS1, S100A7; Pathways effecting cytokine and chemokine expression including, for example, those effecting IL-Iβ, TNF-α, IFN-β, IL-6, IL-la, IL-23a, IL-15, CCL2, CCL19, IL-12; Pathways effecting antigen presentation including, for example, those effecting Sec22B; CD40, CD70, CD86; Pathways effecting cell migration including, for example, those effecting CCR7; Pathways effecting expression of transgenic antigens including, for example, OVA. In some embodiments, the term "XBP1 pathway upregulating composition" refers herein to any composition that when administered to a keratinocyte results in increased innate immune stimulation, increased cytokine/chemokine expression, increased antigen processing and presentation function including those effecting the production of antigen, and/or increased expression or production of one or more of IL-la, IL-Iβ, IFN-β, TNF-a, IL-6, IL-12, IL-15, IL-23a, CCL2,CCL19, MyD88, OAS1, S100A7, Sec22B, CD40, CD70, CD86, CCR7 and HIF-Iα in the keratinocyte.

The term "XBP1" refers herein to an X-box binding protein 1 polypeptide also known as Tax-Responsive Element-Binding Protein 5, TREB5, or XBP2, and in humans, is encoded by the *XBP1* gene. The term "XBP1 polynucleotide" refers to an XBP1 encoding polynucleotide and includes an XBP1 gene in its entirety or a fragment thereof. In some embodiments, the XBP1 polypeptide or polynucleotide is that identified in one or more publicly available databases as follows: HGNC: 12801; Entrez Gene: 7494; Ensembl: ENSG00000100219; OMIM: 194355; and UniProtKB: P17861. In some embodiments, the XBP1 polynucleotide encodes an XBP1 polypeptide comprising the sequence of SEQ ID NO:l, or a polypeptide sequence having at or greater than about 80%, at or greater than about 85%, at or greater than about 90%, at or greater than about 95%, or at or greater than about 98% homology with SEQ ID NO:l, or a polypeptide comprising a portion of SEQ ID NO:l. The XBP1 polypeptide of SEQ ID NO:l may represent an immature or pre-processed form of mature XBP1, and accordingly, included herein are mature

or processed portions of the XBP1 polypeptide in SEQ ID NO: 1. In some embodiments, the XBP1 polynucleotide comprises the sequence of SEQ ID NO:2 or a polynucleotide sequence having at or greater than about 80%, at or greater than about 85%, at or greater than about 90%, at or greater than about 95%, or at or greater than about 98% homology with SEQ ID NO:2, or a polynucleotide comprising a portion of SEQ ID NO:2.

Detailed Description

Provided herein are compositions and methods for increasing an immune response in a subject, immunizing a subject, and/or treating a disease in a subject that relate to keratinocytes. It is a surprising finding of the present invention that modulation of an XBP1 pathway creates a keratinocyte that is itself an immune modulator, or adjuvant. In some embodiments, the concentration of antigen is increased in the vicinity of the keratinocyte, further increasing the immune response by effector cells within that vicinity. Antigen concentration may be increased via increasing production of the antigen by the keratinocyte itself, increasing production of the antigen by another cell, or by administering the antigen to the vicinity. In some embodiments, an antigen encoding polynucleotide is introduced into the keratinocyte and an XBP1 pathway upregulating composition is administered to the keratinocyte to create a keratinocyte that is an antigen-specific immune modulator. These modified keratinocytes produce extracellular antigen, and in some embodiments, pro-inflammatory mediators that facilitate an antigen-specific immune response by immune effector cells. The modified keratinocytes described herein and the compositions contained therein are useful for increasing immune responses, immunizing and treating diseases in subjects.

More specifically, the results provided in herein demonstrate that transient overexpression of XBP1 locally in the skin microenvironment at the time of antigen delivery induces potent local and systemic immune responses. Novel data is provided that demonstrates that inclusion of plasmid DNA encoding XBP1 in skin results in significantly increased durable $CD8^+$ T cell immune responses to a co-delivered transgenic antigen, and, interestingly, accumulated systemic memory and resident $CD8^+$ T cells in the skin at the immunization site. XBP1's expression in the skin is associated with the induction of a pro-inflammatory cutaneous microenvironment, as evidenced by increased expression of pro-inflammatory cytokines and chemokine, and infiltration of lymphocytes and antigen presenting cells. Further, in the *in vitro* systems, it is shown herein that XBP1 has a decisive role in promoting keratinocytes to increase the production of co-delivered secreted antigen and one or more of IL-la, IL-1β, IFN-β, TNF-a, IL-6, IL-12, IL-15, IL-23a, CCL2,CCL19, MyD88, OAS1, S100A7, Sec22B, CD40, CD70, CD86, CCR7 and HIF-1α. The

present disclosure therefore describes for the first time that XBP1 may function as a master transcriptional regulator of keratinocytes to enhance the production of secreted vaccine antigens and pro-inflammatory mediators, resulting in a pro-immunogenic skin microenvironment that enables the induction of robust, durable and effective local and systemic antigen-specific immune responses against cancer or infectious diseases.

Accordingly, provided herein are keratinocytes comprising an XBP1 pathway upregulating composition. In some embodiments, the keratinocytes further comprise an antigen polynucleotide. Included herein are keratinocytes at any stage of differentiation, other than corneocytes. In some embodiments, the keratinocyte expresses or overexpresses a keratin-5 polypeptide and a keratin-14 polypeptide. In other embodiments, the keratinocyte expresses or overexpresses a keratin-1 polypeptide and a keratin-10 polypeptide. In still other embodiments, the keratinocyte expresses or overexpresses a keratin-1 polypeptide and a keratin-16 polypeptide. The keratinocytes produce an increased amount of the antigen extracellularly as compared to a control. In some embodiments, the keratinocytes produce an increased amount of one or more pro-inflammatory mediators extracellularly as compared to a control. In one embodiment, the pro-inflammatory mediators are selected from the group of IL-1a, IL-1β, IFN-β, CCL2, IL-15, IL23a, CCL19, and HIF-1a.

In some embodiments, the XBP1 pathway upregulating composition is a vector comprising an XBP1 DNA polynucleotide. Expression of the XBP1 DNA polynucleotide in the keratinocyte results in overexpression of XBP1 polypeptide as compared to a control, and thereby upregulates the XBP1 pathway. Accordingly, included herein are XBP1 DNA polynucleotides, vectors comprising a XBP1 DNA polynucleotide, and keratinocytes comprising such vectors. However, the XBP1 upregulating composition may be any composition that when administered to a keratinocyte, increases or activates a constituent in an XBP1 regulated/responsive pathway. In some embodiments, the XBP1 upregulating composition increases cytokine/chemokine expression, and /or increases antigen processing and presentation function including those effecting the production of antigen, and/or increases expression or production of one or more of and/or increased expression or production of one or more of IL-1a, IL-1β, IFN-β, TNF-a, IL-6, IL-12, IL-15, IL-23a, CCL2,CCL19, MyD88, OAS1, S100A7, Sec22B, CD40, CD70, CD86, CCR7 and HIF-1α in the keratinocytes.

In those embodiments in which the XBP1 pathway upregulating composition is a vector comprising an XBP1 DNA polynucleotide, the vector may also comprise an antigen DNA polynucleotide. Accordingly, provided herein are vectors comprising an XBP1 DNA polynucleotide and an antigen DNA polynucleotide, and keratinocytes comprising such vectors.

The antigen DNA polynucleotide may be linked to the same or a different promoter as the XBPl DNA polynucleotide. In one embodiment, the XBPl DNA polynucleotide and the antigen DNA polynucleotide are operably linked to the same promoter within the same vector. In other embodiments, the XBPl DNA polynucleotide and the antigen DNA polynucleotide are operably linked to different promoters. The one or more promoters include, but are not limited to, a cytomegalovirus (CMV) promoter, a K14 (keratin 14) promoter, and a CD1 l c promoter. However, it should be understood that the present invention is not limited to the use of a specific promoter. Any promoter that achieves expression of the XBPl DNA polynucleotide within a keratinocyte and/or the antigen DNA polynucleotide within a relevant mammalian cell is within the scope of this invention. In other embodiments, the XBPl DNA polynucleotide and the antigen DNA polynucleotide are contained within different vectors.

In some embodiments, the XBPl polynucleotide encodes an XBPl polypeptide comprising SEQ ID NO: 1 or a homolog or fragment thereof. In some embodiments, the XBPl polynucleotide comprises SEQ ID NO:2 or a homolog or fragment thereof. The antigen polynucleotide of the present invention can be any one known to those of skill in the art. In some embodiments, the antigen polynucleotide encodes a viral antigen. In other embodiments, the antigen polynucleotide encodes a cancer-related antigen. In still other embodiments, the antigen polynucleotide encodes a bacterial antigen.

In other embodiments, the XBPl upregulating composition affects another constituent in the XBPl pathway such as downstream effectors of XBPl including those of the ER stress Gene Network including, for example, Sec24c, Sec31a, Sec23b, Sec24d, Sec61al, Copgl, Copb2, Gosr2, Golgbl, Golga3, Arfgap3, Rpn2, Spcs3, Fasn, Hspal3, Surf4, Jnk, Mfn2, Atf6, Dnajc3, Pdia6, Pdia5, Pdia4, Rpnl, Os9, Hyoul, Csdc47, Stt3a, and Nlrxl. Other downstream pathways known to be effected by XBPl include: Protein transport pathways including, for example, those effecting GRP78; Cell metabolism pathways including, for example, those effecting GFAT-1; Pathways effecting blood vessel growth including, for example, those effecting VEGFA; Pathways effecting triglyceride biosynthesis including, for example, those effecting Agpat6, Fasn, Scd2, or Lparl; Pathways effecting innate immune signaling including, for example, those effecting MyD88, OAS1; Pathways effecting cytokine expression including, for example, those effecting IL-Iβ, TNF-a, IFN-β, IL-6, IL-la, IL-15, CCL2, IL-12; Pathways effecting antigen presentation including, for example, those effecting Sec22B; CD40, CD70, CD86; Pathways effecting cell migration including, for example, those effecting CCR7; Pathways effecting expression of transgenic antigens including, for example, OVA.

Also provided herein are methods of treating or increasing an immune response in a subject by administering to a keratinocyte a pharmaceutically effective amount of one or more compositions comprising an XBPl pathway upregulating composition, wherein the keratinocyte is in the subject or administered to the subject. In these methods, the XBPl pathway upregulating composition, and ultimately the keratinocyte, functions as an adjuvant to increase an immune response in the subject. The XBPl pathway upregulating composition and the keratinocyte used in the methods may be any as described above and below. In some embodiments, the XBPl pathway upregulating composition is administered to keratinocytes within the skin of the subject via a cutaneous, or transdermal administration. In other embodiments, the XBPl pathway upregulating composition is administered to keratinocytes *ex vivo*, and the keratinocytes are then administered to the subject via a form of adoptive cell therapy.

In some embodiments, the method further comprises administering a pharmaceutically effective amount of an antigen to the subject. In these embodiments, the immune response to the antigen is increased, and therefore, these embodiments include methods of immunizing the subject to the antigen. The antigen may be administered before, after or at the same time as the XBPl pathway upregulating composition or keratinocyte comprising the XBPl pathway upregulating composition. As described herein, the antigen may be, but is not limited to, a polypeptide, an oligonucleotide, and a polysaccharide. The antigen may be administered via any means as described herein, and in some embodiments, is administered via a cutaneous, or transdermal administration. In some embodiments, the antigen is administered to a site near or within the vicinity of the keratinocyte.

In some embodiments, an antigen polynucleotide is administered to the keratinocyte. Accordingly, provided herein are methods of immunizing or treating a subject by administering to a keratinocyte a pharmaceutically effective amount of one or more compositions comprising an antigen polynucleotide and an XBPl pathway upregulating composition, wherein the keratinocyte is in the subject or administered to the subject. In some embodiments, the antigen polynucleotide and the XBPl pathway upregulating polynucleotide are administered to keratinocytes within the skin of the subject via a cutaneous, or transdermal administration. In other embodiments, the XBPl pathway upregulating composition and the antigen polynucleotide are administered to keratinocytes *ex vivo*, and the keratinocytes are then administered to the subject via a form of adoptive cell therapy.

Administration of the antigen polynucleotide and an XBPl pathway upregulating composition to the subject results in an immune response in the subject that is specific for the antigen. The antigen specific immune response is mediated, at least in part, by $CD8^+$ T cells. It

is believed that, upon production of the antigen by the keratinocyte, antigen presenting cells present the antigen to $CD8^+$ T cells, thus initiating a $CD8^+$ T cell antigen specific immune response. Any concomitant production of pro-inflammatory mediators by the keratinocyte facilitate this process. In some embodiments, antigen-specific IFN-γ- and Granzyme B-expressing $CD8^+$ T cells are increased locally or systemically in response to the administration. In other or further embodiments, $CD103^+CD8^+$ memory T cells are increased locally or systemically in response to the administration. Since the methods described herein result in both effector and memory T cell responses, the methods of the present invention include use of the compositions described herein for both treatment and immunization.

Treatments according to the invention may be applied preventively, prophylactically, pallatively or remedially. Prophylactic treatments are administered to a subject prior to onset *(e.g. ,* before obvious signs of disease or cancer), during early onset *(e.g. ,* upon initial signs and symptoms of disease or cancer), or after an established development of a disease or cancer. Prophylactic administration can occur for several days to years prior to the manifestation of symptoms of the disease or cancer. In some instances, the terms "treat," "treating," "treatment" and grammatical variations thereof, include partially or completely reducing the size of a solid tumor or a cancerous lesion or reducing the number of solid tumors or cancerous lesions as compared with prior to treatment of the subject or as compared with the incidence of such symptom in a general or study population. Accordingly, the methods of treatment may comprise adoptive cell therapies (ACT) or vaccination therapies.

Included herein is a medicament for increasing an immune response in a subject in need thereof, comprising a pharmaceutically effective amount of a composition comprising an XBPl pathway upregulating composition. Further included is a medicament for treating a viral infection, a bacterial infection or a cancer in a subject, comprising a pharmaceutically effective amount of a composition comprising an XBPl pathway upregulating composition. Still further included herein is a use of an XBPl pathway upregulating composition in the manufacture of a medicament for the treatment of a viral infection, a bacterial infection or a cancer. In each of these embodiments, the XBPl pathway upregulating composition can be as described above and below. In some embodiments, the medicament further comprises an antigen. The antigen can be any as described above and below.

It should also be understood that the foregoing relates to preferred embodiments of the present invention and that numerous changes may be made therein without departing from the scope of the invention. The invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it

is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof, which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims. All patents, patent applications, and publications referenced herein are incorporated by reference in their entirety for all purposes.

EXAMPLES

Example 1: XBPl enhances the production of secreted vaccine antigen and pro-inflammatory cytokines by keratinocytes.

Pam 212 (a mouse keratinocyte cell line) cells (5-6 xlO^4) were cultured overnight in 1 ml DMEM supplemental with 10% FBS, glutamine (2 mM) and 1 **x** antibiotic antimycotic solution and then untreated or transiently co-transfected by plasmid DNA encoding Ova and XBPl (or control vector pcDNA3.1+) using the *TransTT*®-Keratinocyte Transfection Reagent (Mirus) according to vendor's instruction. After 3 days, Ova in the culture supernatants were measured by ELISA (USCN life science Inc., Holzel Diganostik, Germany). The results are shown in Fig. la.

Fig. l(B-D) further shows that XBPl enhances the production of pro-inflammatory cytokines and chemokines by keratinocytes. Experiments were performed as described in relation to Fig. 1A. After 3 days of transfection, IL-la, IFN-β and CCL2 in the culture supernatants were measured by ELISA. Data from four independent experiments are shown in Fig. l(B-D) and were statistically analyzed (Student's *t* test). * P<0.05; ** P<0.01. Notably, in all cases, co-transfection of the dominant-negative XBPl, dnXBPl, which specifically inhibits XBPls without affecting cell viability or growth abrogated these XBPls overexpression effects.

Example 2: XBPl promotes lymphocyte and CDllc $^+$ cell infiltration into skin.

C57BL/6 (B6) mice (6-8 weeks, female) were untreated or immunized with OVA plus control vector DNA or OVA plus XBPl DNA. 3 days later, skin at the immunization site was removed and cut into small pieces and subsequently incubated with Collagenase D (1 mg/ml) and DNase (1 mg/ml) in IMDM for 1 hour in an incubator, and then mashed and passed through a 70 μιη cell strainer. Single-cell suspensions of skin tissues were pre-incubated on ice with Fc Block (BD Bioscience) for 15 minutes and then stained with Fixable Viability Dye EFLUOR® 780, anti-mouse CD45-APC and CDllc-PE-Cy7 and analyzed by flow cytometry using a BD LSRII flow cytometer (BD Biosciences) and analyzed using FlowJo software (v9.2, Tree Star). One

representative of three independent experiments showing $CD45^+$ (Fig. 2A, C, E) or $CDllc^+$ cells (Fig. 2 B, D, F) among total live skin cells is presented in Fig. 2.

Example 3: XBP1 overexpression increases the expression of pro-inflammatory cytokines and chemokines in the skin.

Immunized skin was obtained as described in Example 2. Total RNA was purified from homogenized skin using TRI REAGENT® (Sigma). RNA was quantified by Nanodrop (Thermo Scientific). The cDNA was synthesized from mRNA using The QIAGEN One-Step RT-PCR Kit with gDNA wipeout. Subsequently, TAQMAN® Assay-based real-time PCR was performed in an Applied Biosystems StepOnePlusTM Instrument following standard protocols with primers purchased from IDT DNA for each gene. Mouse β-actin was a control. Relative mRNA expression was determined and normalized based on the $2^{-\Delta\Delta Ct}$ method. Data represent three mice from each group and were statistically analyzed. The results are shown in Fig. 3(A-F).

Example 4: XBP1 overexpression enables induction of durable systemic antigen-specific IFN-γ- and Granzyme B-expressing $CD8^+$ T cell immunity.

B6 mice were untreated or immunized once as described in Example 3. 6-7 weeks later, single-cell suspensions (3×10^6) of splenocytes and vaccine/skin dLN) were restimulated with OVA-specific MHC I peptides (SIINFEKL) (or irrelevant β-gal MHC I peptides: DAPIYTNV) (2 μg/ml) in 2 ml RPMI 1640 10% FBS for 3-4 days. IFN-γ in the culture supernatants was determined by ELISA. Granzyme B was measured by surface staining of anti-mouse CD8-Alexa flour 700 and subsequently intracellular staining of anti-mouse granzyme B-Alexa-647 and analyzed by flow cytometry using a BD LSRII flow cytometer and analyzed using FlowJo software. One representative of three independent experiments showing Granzyme B expression in gated $CD8^+$ T cells is presented in Fig. 4(A-E).

Example 5: XBP1 promotes the accumulation of memory lcentral ($CD44^+CD62L^+$) and effector ($CD44^+CD62L^-$)1 $CD8^+$ T cells and skin-resident $CD103^+CD8^+$ memory T cells in skin at the immunization site.

Mice were immunized as described in Example 4. 6-7 weeks later, single-cell suspensions from skin at the immunization site were prepared as described in Example 2, and subsequently pre-incubated on ice with Fc Block for 15 minutes and then stained with Fixable Viability Dye EFLUOR® 450 (dead cells were excluded from analysis), anti-mouse CD45-APC, CD8-PE-Cy7, CD44-FITC, CD62L-percep5.5, CD103-PE, and analyzed by flow cytometry using a BD LSRII

flow cytometer and analyzed using FlowJo software. One representative of 3 experiments showing $CD44^+CD62L^+$ and $CD44^+CD62L^-$ in gated $CD8^+$ T cells or $CD103^+CD8^+$ T cells in gated total live skin cells is shown in Fig. 5.

Example 6: XBPl increases pro-inflammatory cytokines and chemokines in human skin *in situ*.

Human skin epidermal/dermal explants were left untreated or immunized with human XBPl or control vector and cultured. 72 hours later, skin at the immunization site was used for RNA extraction. Relative expression of various genes was determined by real-time qRT-PCR and normalized based on the $2^{-\Delta\Delta Ct}$ method. The human B2M housekeeping gene served as an internal control.

Data from three experiments using three different human skin explants is presented individually in Fig. 6(A-0), which shows increased expression of XBPl, GRP78, GFAT-1, VEGFA, HIF-la, IL-Iβ, MyD88 , IL-23a, OAS1, S100A7, TNF-a, CCL19, CD86, IL-15, and OVA.

Example 7: Transient overexpression of XBPls in skin drives vaccine-induced durable protective immunity .

B6 mice (4/group) were untreated or immunized once as in Example 4. 5 months later, mice were i.d. challenged with exponentially growing B16-OVA ($lxlO^5$). Melanoma growth was monitored and data were statistically analyzed (9). * $P<0.05$. The results are shown in Fig. 7.

SEQUENCES

SEQ ID NO:l

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361 anelfpqlis v

SEQ ID N0:2

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961 ctgagaccac cttcttgcct gctggacgct cacagtgact gtggatatga gggctcccct
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1081 gccaatgaac ttttccccca gctgattagt gtctaa

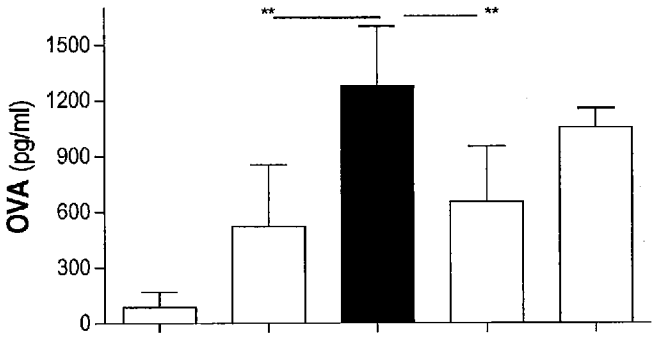
CLAIMS

1. A method of increasing an immune response in a subject in need thereof, comprising administering to a keratinocyte a pharmaceutically effective amount of a composition comprising an XBPl pathway upregulating composition, wherein the keratinocyte is in the subject or administered to the subject.
2. A method of treating a viral infection, a bacterial infection or a cancer in a subject, comprising administering to a keratinocyte a pharmaceutically effective amount of a composition comprising an XBPl pathway upregulating composition, wherein the keratinocyte is in the subject or administered to the subject.
3. The method of claim 1 or 2, wherein the subject has a skin infection or a skin cancer.
4. The method of any one of claims 1-3, further comprising administering a pharmaceutically effective amount of an antigen to the subject.
5. The method of claim 4, wherein the antigen is a bacterial antigen.
6. The method of claim 4, wherein the antigen is a viral antigen.
7. The method of claim 4, wherein the antigen is a cancer-related antigen.
8. The method of any one of claims 1-7, wherein the XBPl pathway upregulating composition is a vector comprising an XBPl polynucleotide.
9. The method of claim 8, wherein the XBPl polynucleotide encodes a polypeptide comprising SEQ ID NO:l or a homolog thereof.
10. The method of claim 4, wherein the XBPl polynucleotide comprises SEQ ID NO:2.
11. The method of any one of claims 4-7, wherein the antigen is a polypeptide.
12. The method of claim 11, wherein the XBPl pathway upregulating composition is a vector comprising an XBPl polynucleotide and the vector further comprises an antigen polynucleotide.
13. The method of any one of claims 2-12, wherein the subject is treated prophylactically.
14. The method of any one of claims 1-13, wherein administration is cutaneous or transdermal.
15. A polynucleotide comprising an XBPl polynucleotide operably linked to a K14 promoter and an antigen polynucleotide operably linked to a promoter.
16. A vector comprising the polynucleotide sequence of claim 15.
17. A keratinocyte comprising the polynucleotide sequence of claim 15 or the vector of claim 16.

18. The keratinocyte of claim 17, wherein the one or more pro-inflammatory mediators is selected from the group consisting of IL-la, IL-Iβ, IFN-β, CCL2, IL-15, IL23a, CCL19, and HIF-Iα and/or various cell function associated genes including GRP78, GFAT-1, TNF-a, VEGFA, HIF-la, OASl, S100A7, CD86, and/or delivered vaccine antigen OVA.
19. A medicament for increasing an immune response in a subject in need thereof, comprising a pharmaceutically effective amount of a composition comprising an XBP1 pathway upregulating composition.
20. A medicament for treating a viral infection, a bacterial infection or a cancer in a subject, comprising a pharmaceutically effective amount of a composition comprising an XBP1 pathway upregulating composition.

FIGURE 1

A.



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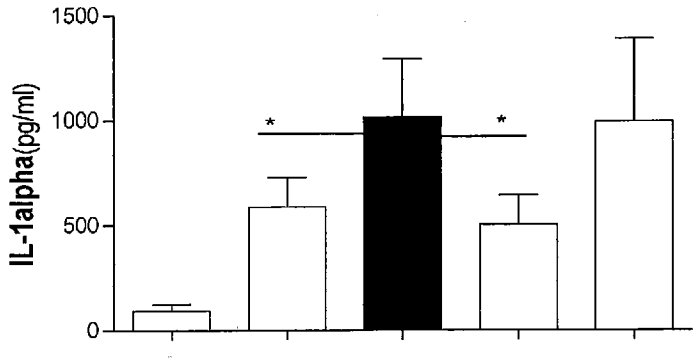
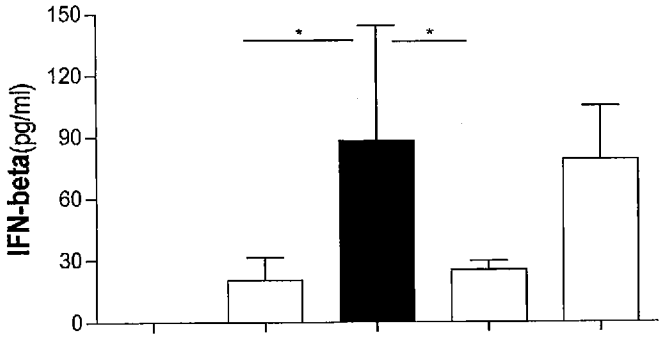


FIGURE 1 CONTINUED

C.



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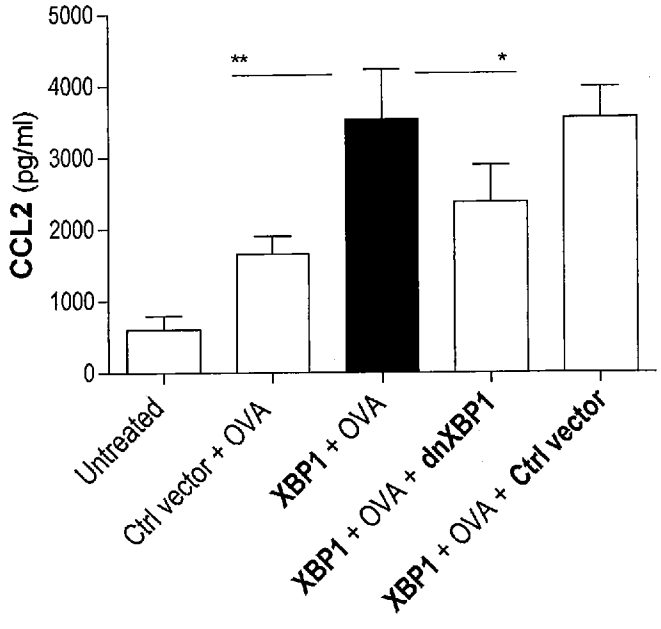


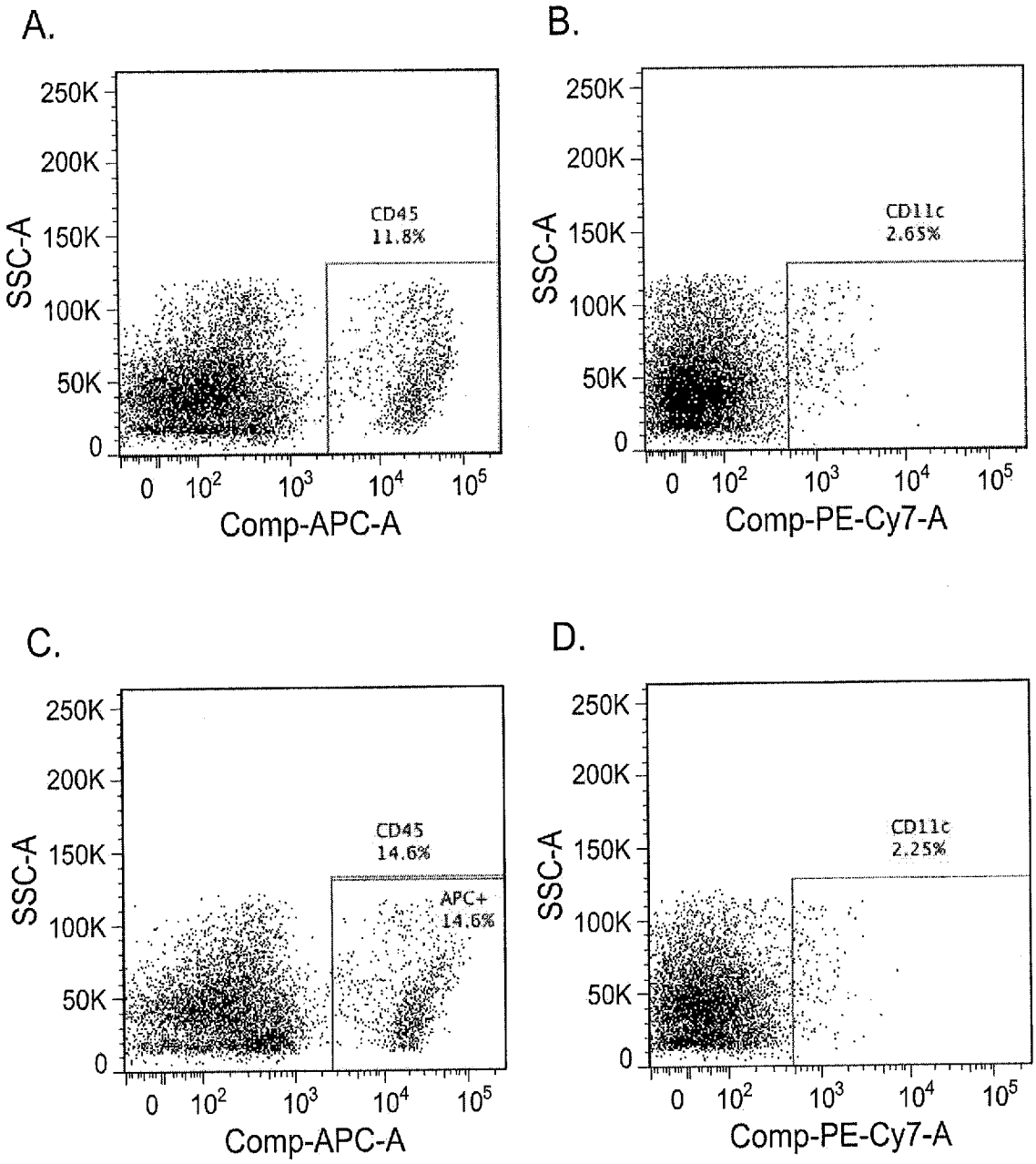

A.
B.
C.
D.
SSC-A
Comp-APC-A
Comp-PE-Cy7-A
250K
200K
150K
100K
50K
0
10^2
10^3
10^4
10^5
CD45
11.8%
CD11c
2.65%
CD45
14.6%
APC+
14.6%
CD11c
2.25%

FIGURE 2

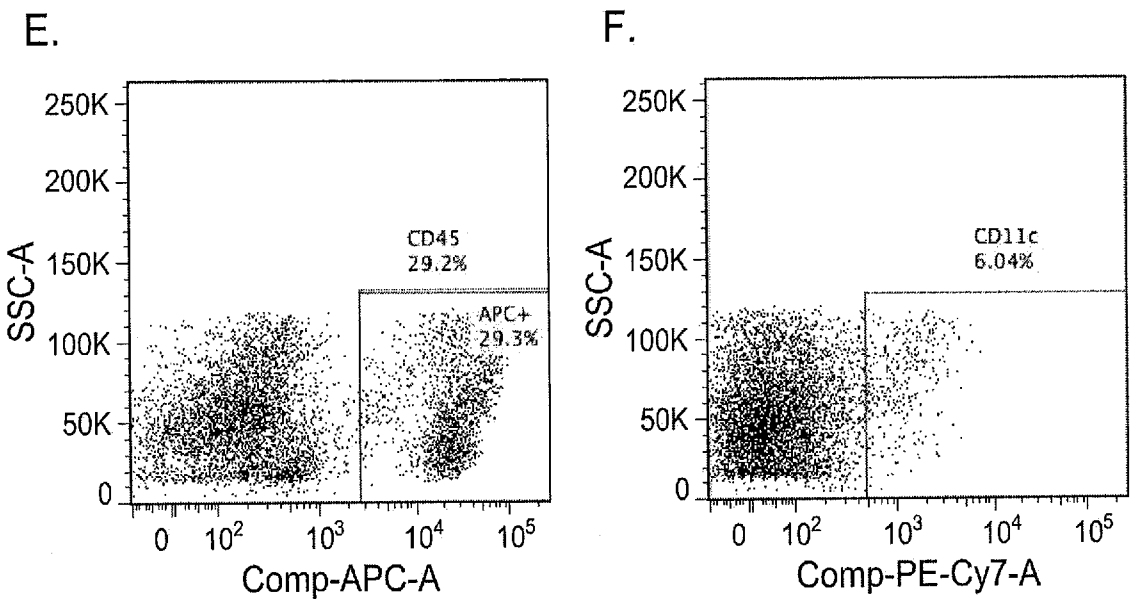
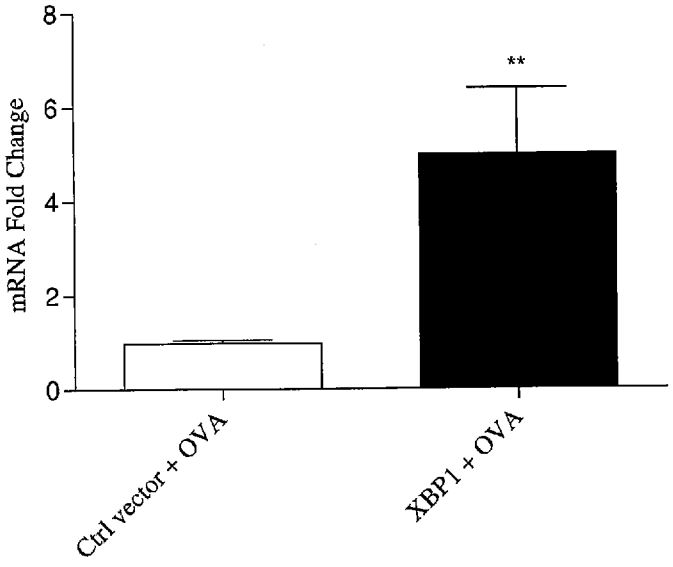


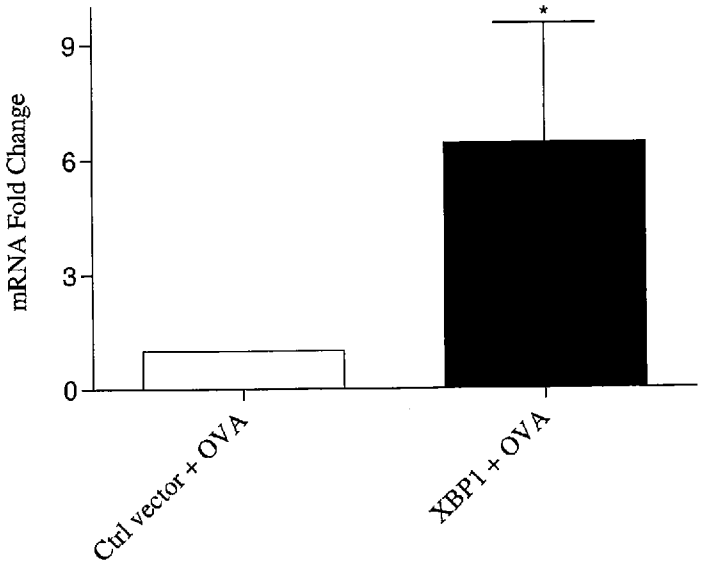
FIGURE 2 CONTINUED

FIGURE 3

A.


8
6
4
2
0
mRNA Fold Change
**
Ctrl vector + OVA
XBP1 + OVA

B.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6
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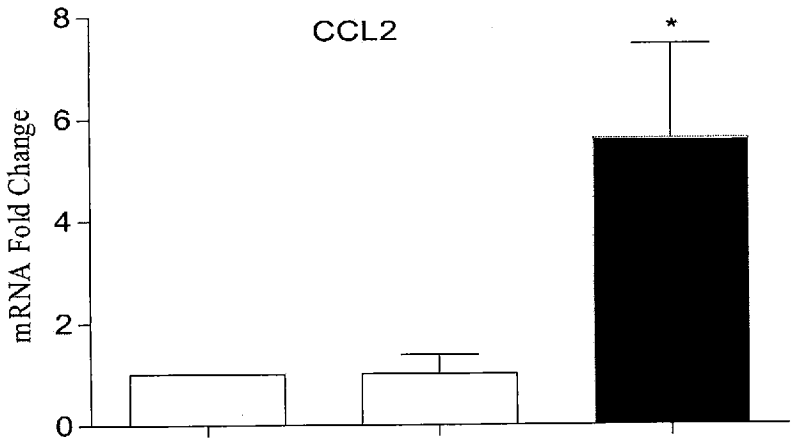
mRNA Fold Change
*
Ctrl vector + OVA
XBP1 + OVA

FIGURE 3 CONTINUED

C.



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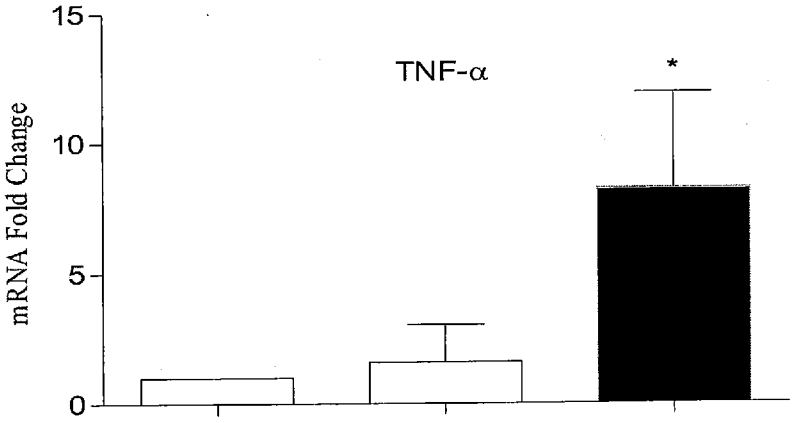
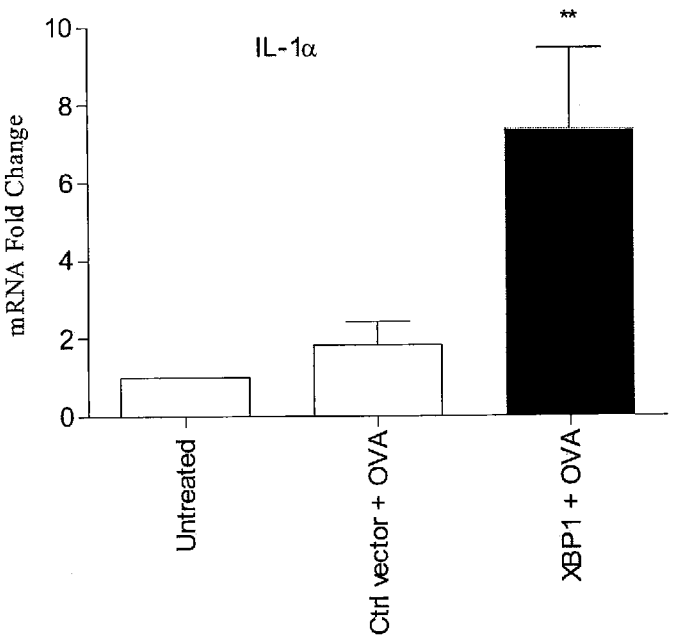
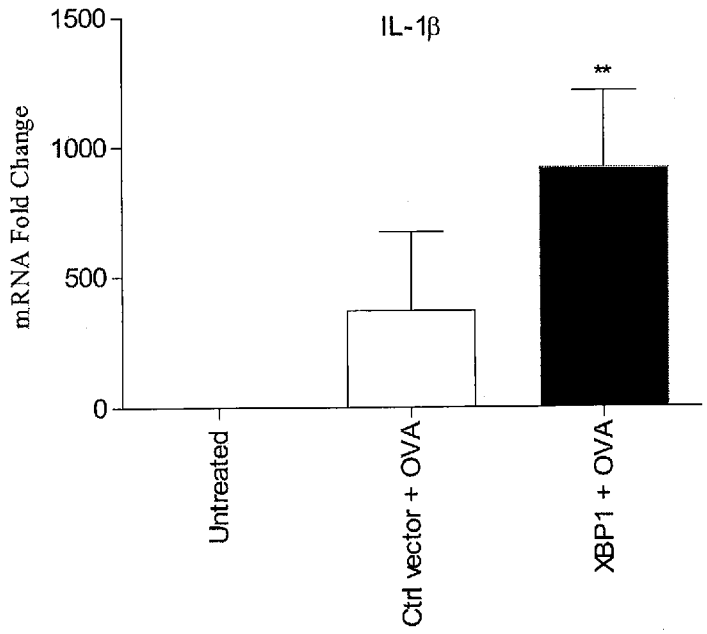


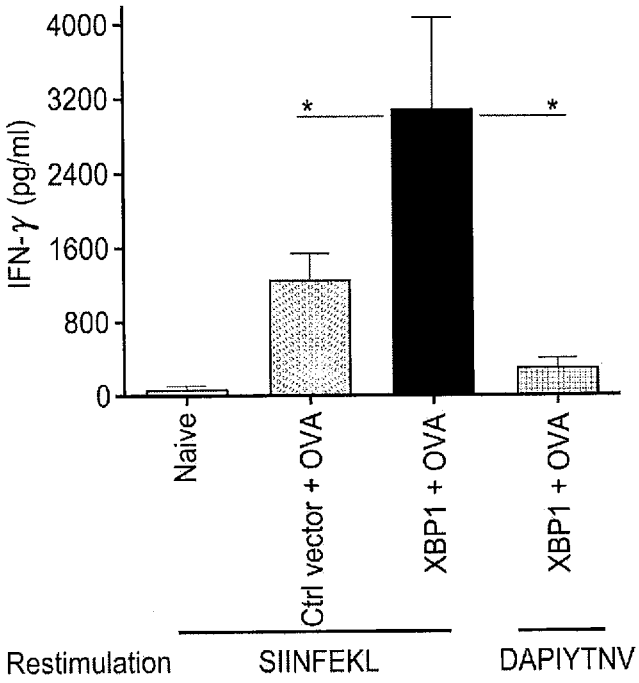
FIGURE 3 CONTINUED

E.



F.




4000
3200
2400
1600
800
0
IFN-γ (pg/ml)
*
*
Naive
Ctrl vector + OVA
XBP1 + OVA
XBP1 + OVA
Restimulation
SIINFEKL
DAPIYTNV

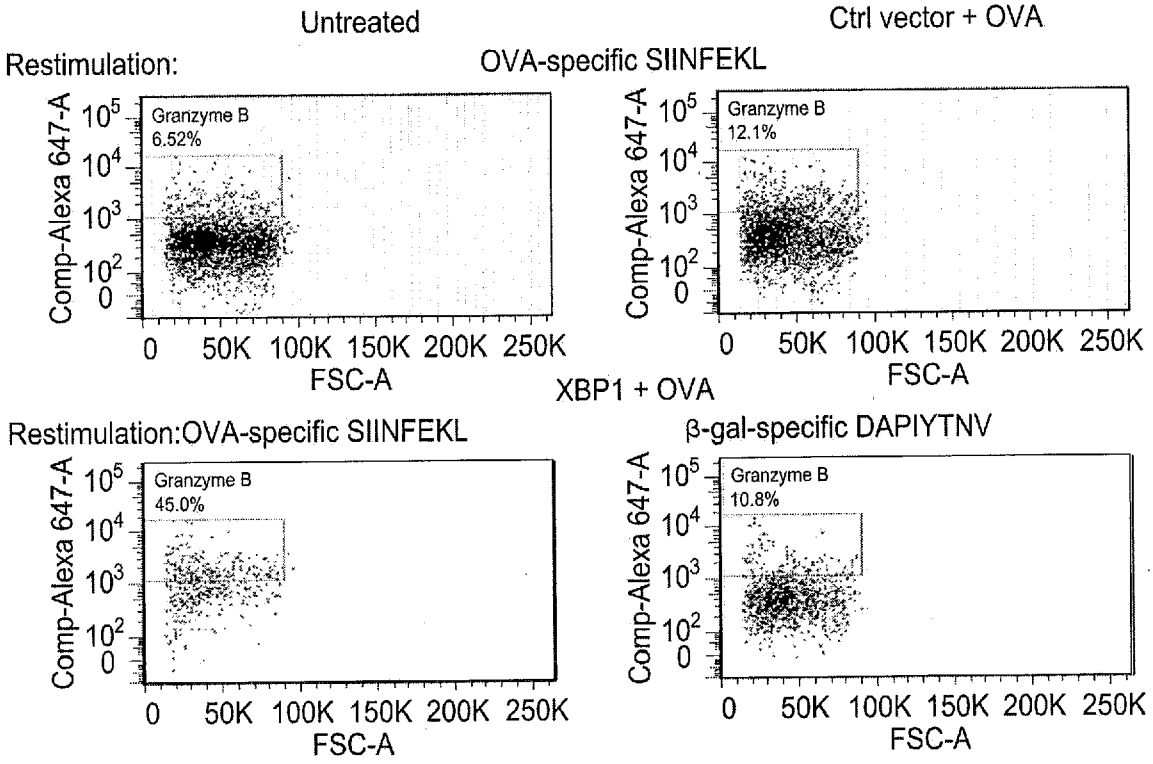

Untreated
Ctrl vector + OVA
Restimulation:
OVA-specific SIINFEKL
Comp-Alexa 647-A
Granzyme B
6.52%
Granzyme B
12.1%
FSC-A
XBP1 + OVA
Restimulation:OVA-specific SIINFEKL
β-gal-specific DAPIYTNV
Granzyme B
45.0%
Granzyme B
10.8%

FIGURE 4

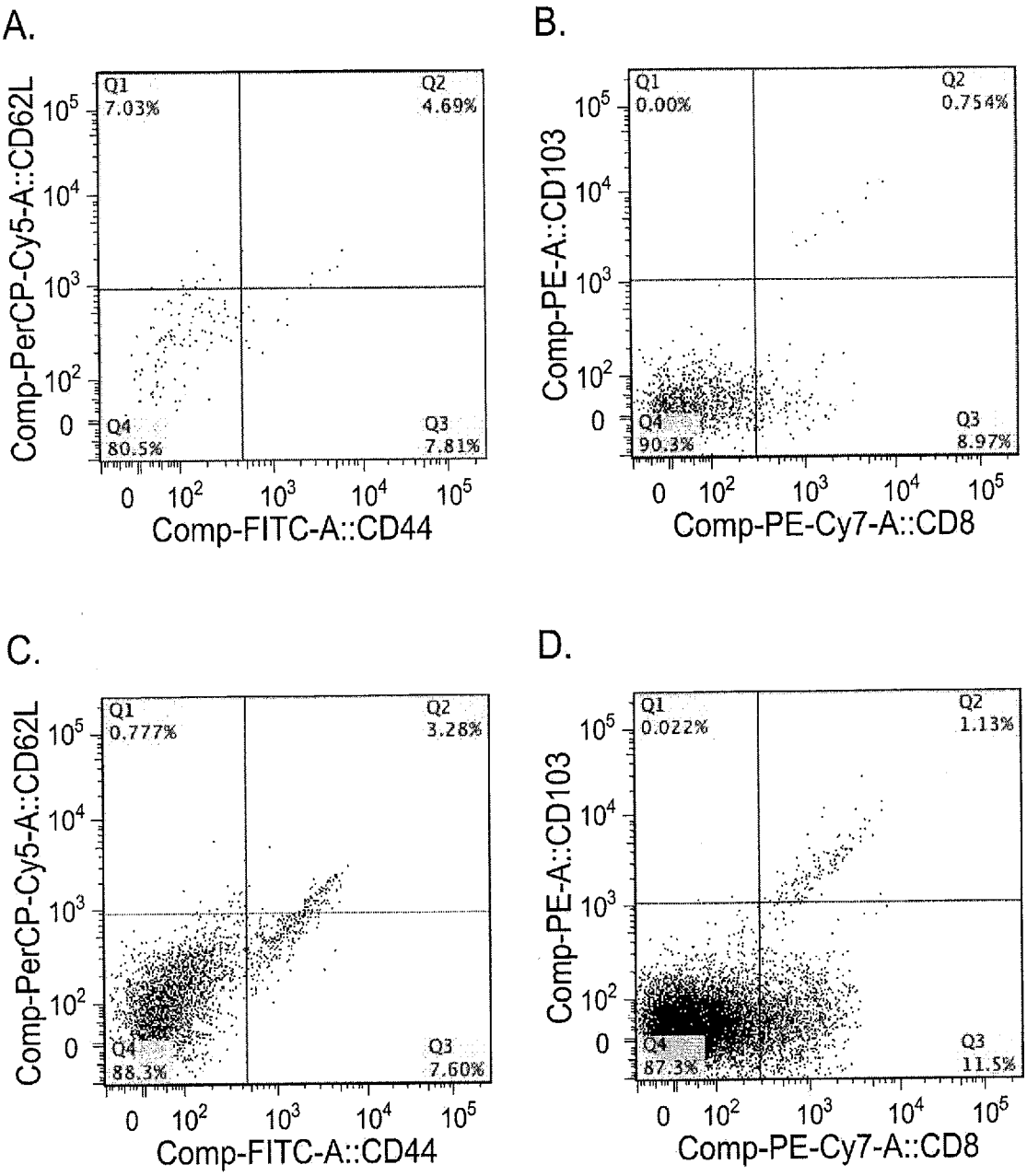
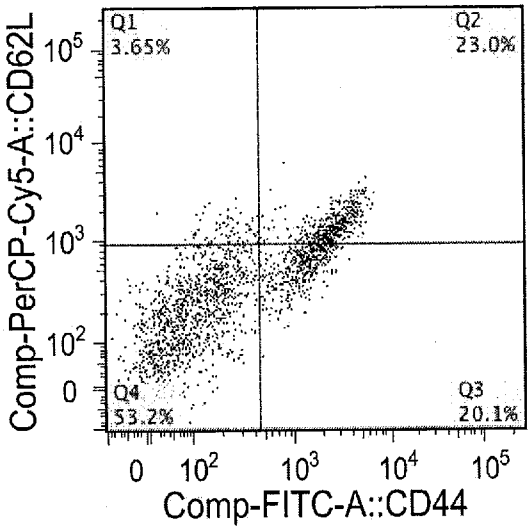

A.
Q1
7.03%
Q2
4.69%
Q4
80.5%
Q3
7.81%
Comp-PerCP-Cy5-A::CD62L
Comp-FITC-A::CD44
B.
Q1
0.00%
Q2
0.754%
Q4
90.3%
Q3
8.97%
Comp-PE-A::CD103
Comp-PE-Cy7-A::CD8
C.
Q1
0.777%
Q2
3.28%
Q4
88.3%
Q3
7.60%
Comp-PerCP-Cy5-A::CD62L
Comp-FITC-A::CD44
D.
Q1
0.022%
Q2
1.13%
Q4
87.3%
Q3
11.5%
Comp-PE-A::CD103
Comp-PE-Cy7-A::CD8

FIGURE 5

E.



F.

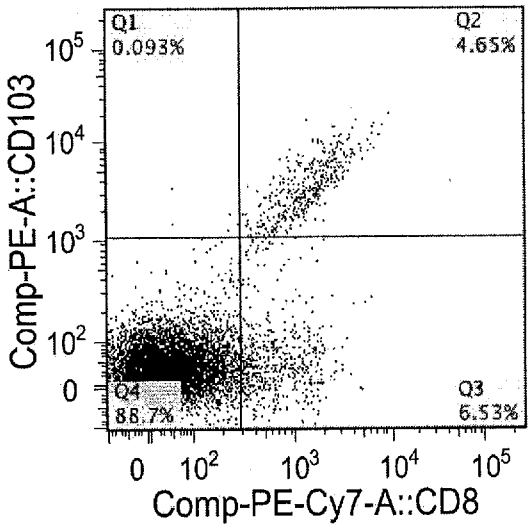
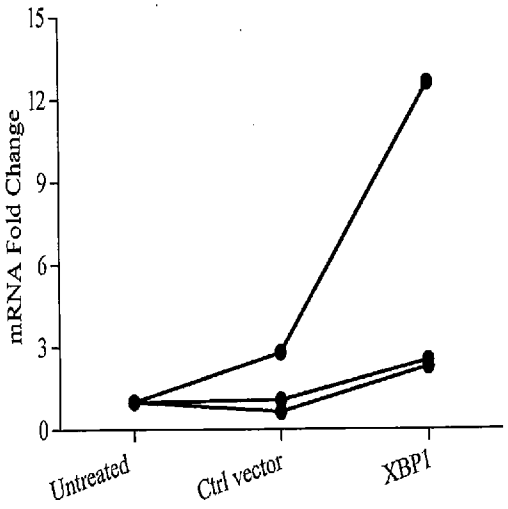


FIGURE 5 CONTINUED

FIGURE 6

A.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12
9
6
3
0
mRNA Fold Change
Untreated
Ctrl vector
XBP1

B.

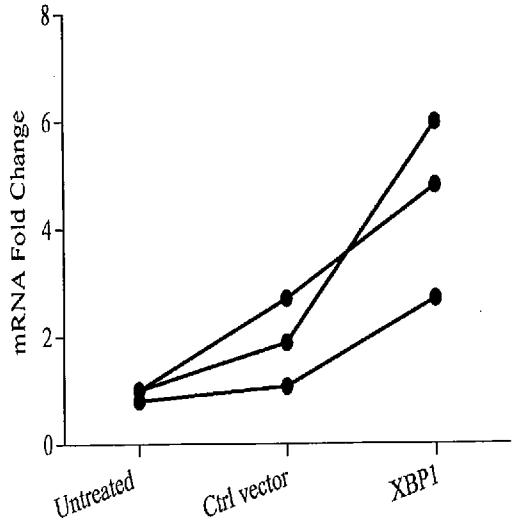
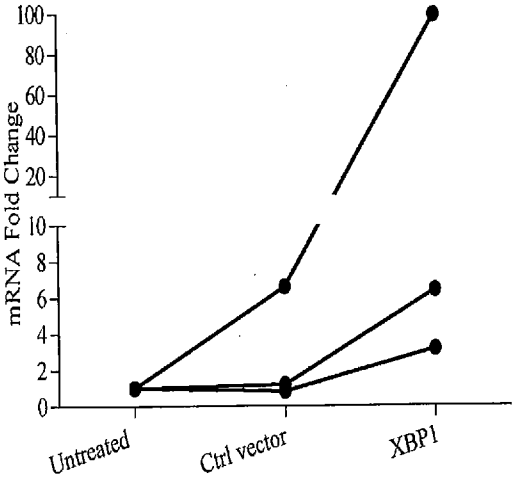

8
6
4
2
0
mRNA Fold Change
Untreated
Ctrl vector
XBP1

FIGURE 6 CONTINUED

C.



D.

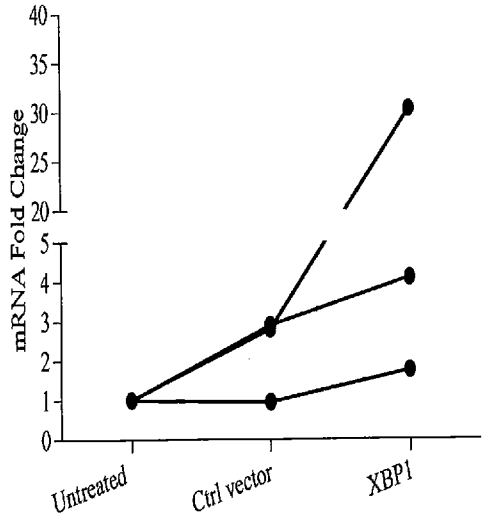


FIGURE 6 CONTINUED

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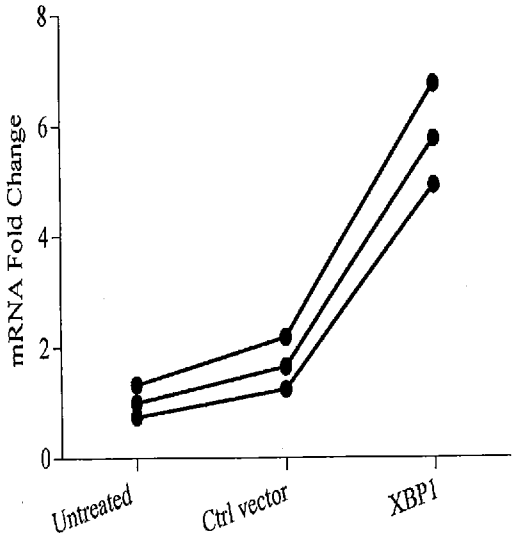

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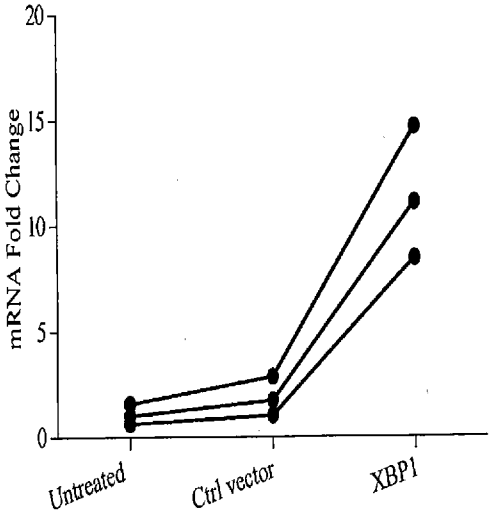

FIGURE 6 CONTINUED

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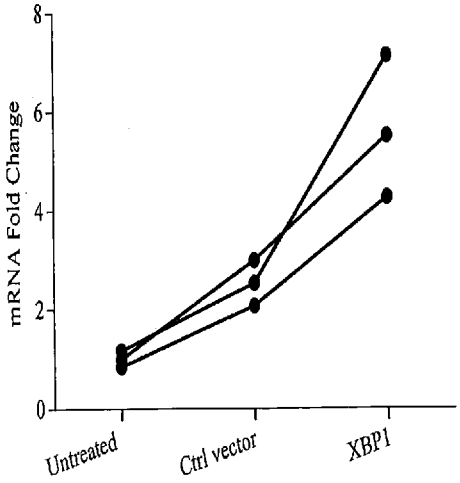

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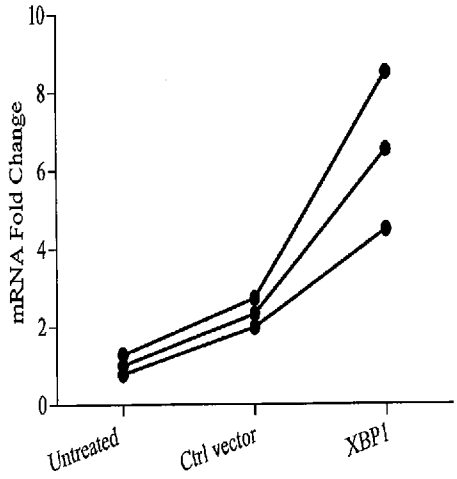

FIGURE 6 CONTINUED

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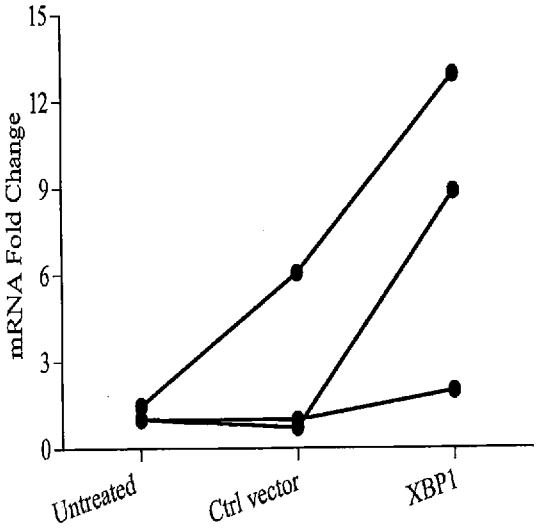

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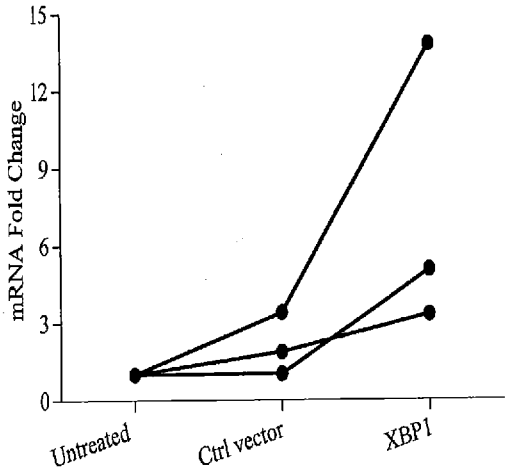
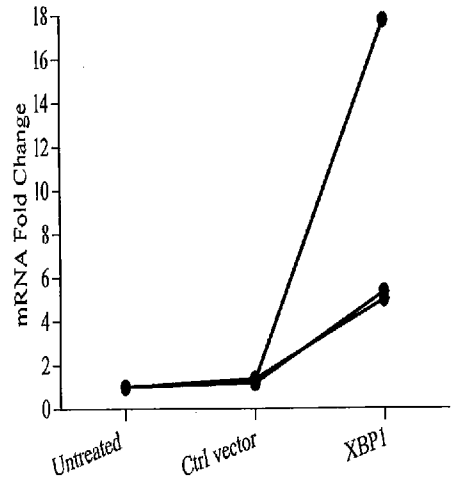


FIGURE 6 CONTINUED

K.



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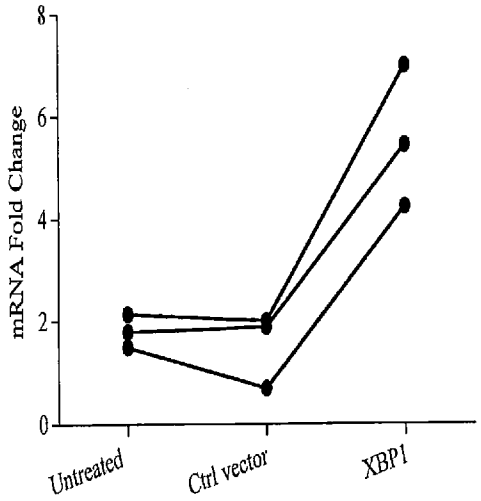

FIGURE 6 CONTINUED

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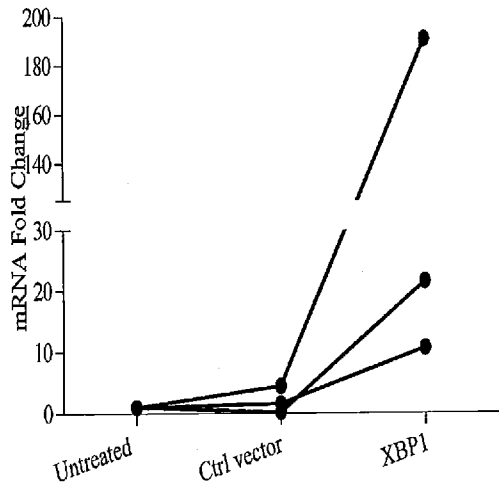


N.

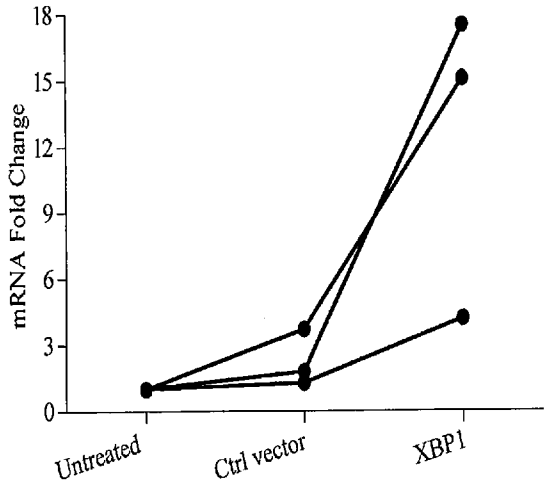


FIGURE 6 CONTINUED

O.

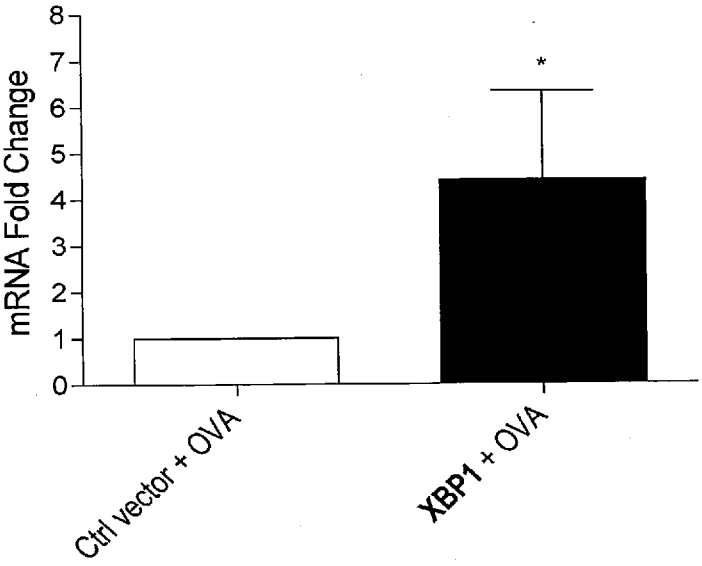
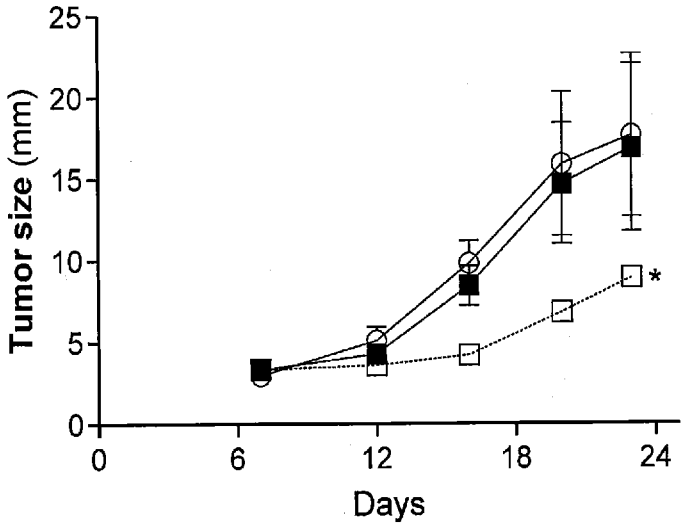


FIGURE 7



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/039265

A. CLASSIFICATION OF SUBJECT MATTER

A61K 38/17 (2006.01) A61K 48/00 (2006.01) C07K 14/47 (2006.01) A61P 31/12 (2006.01) A61P 37/02 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

Databases consulted: WPIAP, EPODOC, MEDLINE, EMBASE, BIOSIS, HCAPLUS, PATENSCOPE, ESPACENET, PUBMED

Search terms: XBOX binding protein; XBPl; Tax responsive element binding protein; TREB5; XBPl ; RFX peptide; RFXl; RFX3; keratinocyte; promote, regulate, express, immune, inflammation, IL-2; IFN; CCL2; CCL1 9; HIF; GRP78; virus, viral, bacteria, cancer, carcinoma, tumour, metastasis, vector, nucleotide, antigen, immunogen, Louis Falo, Zhaoyang Yao (inventor search) and related terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 August 201 6	24 August 2016
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Safiea Goolam AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262832521

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item l.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

   There was a sequence listing filed but it was not used for the purpose of this search and opinion.

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHANG YI, et al. 'Genetic Vaccines To Potentiate the Effective CD103+ Dendritic Cell-Mediated Cross-Priming of Antitumour Immunity,' *The Journal of Immunology*, (published online 13 May 2015), vol 194, pp 5937-5947. Abstract; page 5939 column 1; Table 2 at page 5941; page 5945 column 1	1-20
X	WO 2004/111194 A2 (AILOR EN, REEF ME.) 23 December 2004 Abstract; claims 28-29	19-20
X	WO 2010/008860 A1 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 21 January 2010 Abstract; [0044]-[0045]	19-20

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2016/039265**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2004/111194 A2	23 December 2004	WO 2004111194 A2	23 Dec 2004
		AU 2004248165 A1	23 Dec 2004
		CA 2528418 A1	23 Dec 2004
		EP 1639095 A2	29 Mar 2006
		JP 2007500016 A	11 Jan 2007
		NZ 544335 A	31 Jan 2009
		US 2005106222 A1	19 May 2005
WO 2010/008860 A1	21 January 2010	WO 2010008860 A1	21 Jan 2010
		US 2011142799 A1	16 Jun 2011

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.