

BRIEF SUMMARY

[0005a] In one aspect, the present invention provides a method of obtaining information that can be used to determine the immunogenicity of antigens for a pathogen or cancer, comprising the steps of:

exposing cells in vitro to more than one antigen from a pathogen or cancer under conditions to allow cells that recognize the antigens to expand in quantity, wherein the cells are to be obtained from a plurality of individuals,

adding one of said antigens into separate cultures of the expanded cells,

comparing a determined magnitude of a biological activity to a threshold value to determine if the antigen is a responder or a non-responder, and

identifying the antigen as a responder if the biological activity exceeds the threshold.

[0005b] In another aspect, the present invention provides a method of producing a T cell culture, comprising the steps of:

exposing PBMCs and/or T cells from a plurality of individuals to a part of a pathogen or tumor or a complete pathogen or tumor for at least 48 hours; followed by

re-challenge of the cells to individual antigens of the same pathogen or tumor.

[0006] Examples of the disclosure concern methods, compositions, and/or systems for generating immunotherapies, including for identifying one or more antigen(s) that would be clinically efficacious in one or more immunotherapies for a mammalian individual, such as a human or a dog. Examples of the methods concern determining which antigens from a pathogen or cancer cell are suitably immunogenic to be utilized for one or more therapeutic applications in a mammal. In certain examples the pathogen is a virus, bacteria, or fungi, for example.

[0007] In particular examples, methods of the disclosure concern the determination of the immunogenicity for a plurality of antigens from a pathogen and/or cancer cell in question and concern an ordering of the immunogenicity based on a suitable biological determination. The determination of a hierarchy of the antigens based on their immunogenicity

can be utilized in considering a suitable immunogenic therapy for an individual that has the pathogen or cancer and that is susceptible or at risk for exposure and/or disease from the pathogen and/or cancer. In specific examples, the suitable immunogenic therapy comprises the most suitable immunogenic therapy based on the immunodominance of antigens for a particular pathogen or cancer.

[0008] In various examples at least two antigens from a pathogen or cancer are identified and their level of immunogenicity is quantitated. The antigens can then be ranked in order based upon their level of immunogenicity so that a determination can be made as to which of the antigens should be used a target for an immunotherapy.

[0009] The immunogenic therapy designed based on the determination of the hierarchy of antigens may be of any kind, but in specific examples the immunogenic therapy comprises vaccines, adoptive cell therapy, monoclonal antibody, bi-specific antibodies (BITES), oncolytic virus, or a combination thereof. The immunotherapy may comprise adoptive T cell therapy (native T cell receptor (TCR), engineered TCRs, or CAR T cells), although other types of immune cells may be utilized, including NK cells, NK T cells, mesenchymal stromal cells (MSCs), and dendritic cells (DC). The immunotherapy may rely on the production and use of immune cells including those that rely on T cell receptors that are native or engineered and those that rely on NK cells, NKT cells, mesenchymal stromal cells (MSCs), and dendritic cells (DCs). For example, a T cell therapy can be designed that attacks at least two of most immunogenic

antigens of a pathogen or cancer with the intent of destroying a respective pathogenic cell or a cancer cell. Such a therapy could include a T cell manufacturing process that generates T cells that are able to recognize at least two of the most immunogenic antigen targets from a pathogen or cancer. In alternative examples, the T cell therapy targets only one antigen from a pathogen or cancer.

[0010] In certain examples, the immunogenicity of more than one antigen from a pathogen or a cancer is determined and is ordered into a hierarchy for use in considering the one or more antigens to be utilized in therapy for an individual that has been exposed to the pathogen (including has contracted a disease caused directly or indirectly by the pathogen), or may be exposed to the pathogen, or who has cancer or is at risk of developing cancer, or as a result of being exposed to the pathogen is at risk of developing cancer.

[0011] In specific examples, evaluations are undertaken to build an information base that can be used to assign a value to the level of immunogenicity of a given antigen. The information base is established as follows: a) peripheral blood mononuclear cells (PBMCs), obtained from pathogen exposed individuals or cancer patients, undergo an in vitro culture process that exposes the PBMCs to two or more antigens from the pathogen or two or more antigens from the cancer, preferably in the presence of Th-1 polarizing cytokines; b) a period of time is allowed to let T cells that recognize the antigens multiply in quantity; c) the culture is split into as many separate cultures as the number of antigens that were present in step a and only one of the antigens is added into each culture, each culture thereby including one of the original antigens and each culture including an antigen that differs from the other cultures; d) biological activity is measured (which may be referred to as a biological measurement); e) either in parallel or at a different time, perhaps becoming a historic reference, a threshold for biological activity is established by an in vitro assessment of the biological activity occurring in PBMCs that have not been exposed sequentially or in parallel in vitro to the same agent(s) of step c; f) the biological activity of each of the cultures of step c is compared to the threshold and those that have biological activity that exceeds the threshold are classified as responders and those that do not have biological activity that exceeds the threshold are classified as non-responders and g) as a result of steps a-f the frequency of responders and non-responders relative to the number individuals evaluated is known and the magnitude of the responders biological activity relative to the threshold and relative to zero is known.

[0012] Skilled artisans recognize that in specific aspects a threshold is the noise in the process of measuring biological activity. There is more than one way to establish the threshold. For example, as described above, PBMCs can be exposed to each antigen used in the process and the subsequent biological activity can be measured to establish the threshold. Alternatively for example, the cultures of step c can also be challenged with an antigen that is not included in step a. Both approaches will detect noise and thereby establish the threshold that needs to be exceeded for the culture that is exposed to the antigen of interest to be classified as a responder.

[0013] In certain examples, there is a method of characterizing immunogenic antigens for a pathogen or cancer, comprising the steps of providing or generating multiple pluralities of T cells, wherein each plurality of T cells is specific for one antigen from the pathogen or cancer; assaying for a quantifiable biological response from cells from each plurality upon challenge from their respective antigens, wherein a quantitated output of the biological response is measured against a threshold quantity to determine the efficacy and magnitude of the response from each plurality, wherein the efficacy is the percentage of individuals in a population that have a biological response that exceeds the threshold quantity; and ordering the immunogenicity of each antigen based on a mathematical relationship between the efficacy and magnitude of the response from each plurality.

[0014] In other examples, a formula is established that uses variables including the percentage of responders and the percentage of non-responders as well as the magnitude of the biological response to assign a value to an antigen, the higher the value the higher the level of immunogenicity assigned to a particular antigen.

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[0016] Provided herein are examples of methods of immune profiling of exemplary Virus X, Bacteria Y, Tumor Z and also of specific but exemplary viruses, such as parainfluenza type III (PIV3) and human Metapneumovirus (hMPV). In specific examples, the immunogenicity of multiple antigens for PIV3 and hMPV are respectively determined and utilized in therapy.

[0017] In particular examples, the methods and compositions of the disclosure concern adoptive T-cell therapy to prevent or treat infection. such as following HSCT. In particular cases, the disclosure includes therapy for the prevention or treatment of viral infection, including at least for infection by PIV (including at least PIV3), hMPV, cytomegalovirus, BK virus, human herpesvirus 6, adenovirus, Epstein Barr virus, BK virus, RSV, Influenza, Parainfluenza, Bocavirus, Coronavirus, LCMV, Mumps, Measles, Metapneumovirus, Parvovirus B, Rotavirus, West Nile Virus, JC, or HHV7, for example. Viruses from any viral infection may be treated, including from one of the following families: *Adenoviridae*, *Picornaviridae*, *Herpesviridae*, *Hepadnaviridae*, *Flaviviridae*, *Retroviridae*, *Orthomyxoviridae*, *Paramyxoviridae*, *Papovaviridae*, *Polyomavirus*, *Rhabdoviridae*, or *Togaviridae*.

[0018] Bacteria that may comprise the pathogen of interest include at least the following: *Bacillus anthracis*, *Bacillus cereus*, *Bartonella henselae*, *Bartonella Quintana*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Borrelia garinii*, *Borrelia afzelii*, *Borrelia recurrentis*, *Brucella abortus*, *Brucella canis*, *Brucella melitensis*, *Brucella suis*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydophila psittaci*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Clostridium tetani*, *Corynebacterium diphtheria*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Francisella tularensis*, *Haemophilus influenza*, *Helicobacter pylori*, *Legionella pneumophila*, *Leptospira interrogans*, *Leptospira santarosai*, *Leptospira weilii*, *Leptospira noguchii*, *Listeria monocytogenes*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycobacterium ulcerans*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitides*, *Pseudomonas aeruginosa*, *Rickettsia rickettsii*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Yersinia pestis*, *Yersinia enterocolitica*, or *Yersinia pseudotuberculosis*, for example.

[0019] In particular examples, the methods and compositions of the disclosure also concern adoptive T-cell therapy to prevent or treat cancer, including cancer that is selected from the group consisting of Acute Lymphoblastic Leukemia (ALL), acute Myeloid Leukemia (AML), Adrenocortical Carcinoma, Adrenal Cortex Cancer, AIDS-Related Cancers, Kaposi Sarcoma, AIDS-Related Lymphoma, Primary CNS Lymphoma, Anal Cancer, Appendix Cancer, Astrocytomas, Atypical Teratoid/Rhabdoid Tumor, Basal Cell Carcinoma, Bile Duct Cancer,

CLAIMS

What is claimed is:

1. A method of obtaining information that can be used to determine the immunogenicity of antigens for a pathogen or cancer, comprising the steps of:
exposing cells *in vitro* to more than one antigen from a pathogen or cancer under conditions to allow cells that recognize the antigens to expand in quantity, wherein the cells are to be obtained from a plurality of individuals, adding one of said antigens into separate cultures of the expanded cells, comparing a determined magnitude of a biological activity to a threshold value to determine if the antigen is a responder or a non-responder, and identifying the antigen as a responder if the biological activity exceeds the threshold.
2. The method of claim 1, wherein the pathogen is a virus, fungus, or bacteria.
3. The method of claim 1 or 2, wherein the biological activity comprises production of IFN γ , IL2, Granzyme B, GM-CSF, and/or TNF α .
4. The method of any one of claims 1-3, further comprising the step of determining the threshold value.
5. The method of any one of claims 1-4, wherein the mathematical formula is the following:
$$\frac{(\% \text{ of responders} + 1)}{(\% \text{ of non-responders} + 1)} \times \frac{\text{Magnitude of Response}}{}$$
6. The method of claim 2, wherein the virus is a respiratory virus.
7. The method of claim 6, wherein the respiratory virus is a parainfluenza virus or metapneumavirus.
8. The method of claim 7, wherein the parainfluenza virus is type III.
9. The method of claim 7, wherein the metapneumavirus is human metapneumavirus.