

SUMMARY

[0005a] In one example, the present disclosure 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 example, the present disclosure 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.

[0005c] In one aspect, the present disclosure refers to a method of establishing a hierarchy of immunogenicity of antigens for a pathogen or cancer, comprising the steps of:

(a) exposing peripheral blood mononuclear cells (PBMCs) obtained from an individual to two or more antigens from a pathogen or cancer in an *in vitro* culture under conditions to allow T cells in the PBMCs that recognize the antigens to expand in quantity to produce expanded T cells,

(b) splitting the expanded T cells into a plurality of separate cultures and adding only one of said antigens into each of the separate cultures;

(c) measuring the magnitude of response of a biological activity that results from the addition of each respective antigen to each of the separate cultures,

(d) comparing the magnitude of response of the biological activity from each of the separate cultures to a threshold value to discriminate expanded T cell cultures that are responders from T cell cultures that are non-responders, wherein the threshold value is established by challenging the expanded T cells with an antigen that was not included in step (a);

identifying each of the separate expanded T cell cultures as responders to the antigen added in step (c) if the biological activity exceeds the threshold and as non-responders to the antigen if the biological activity does not exceed the threshold,

performing the same method using PBMC samples obtained from multiple individuals;

for each antigen, determining the frequency, relative to the number of individuals evaluated, that the expanded T cell cultures are responders and non-responders; and

establishing a hierarchy of immunodominance of the antigens based on a mathematical formula:

$$\frac{(\% \text{ of responders} + 1) \times \text{Magnitude of Biological Response}}{(\% \text{ of non-responders} + 1)}.$$

[0005d] In another aspect, the present disclosure refers to a method of obtaining information for determining the immunogenicity of antigens for a pathogen or cancer, comprising the steps of:

(a) exposing T cells from an individual *in vitro* to more than one antigen from a pathogen or cancer under conditions to allow T cells that recognize the antigens to expand in quantity,

(b) splitting the T cells that expanded in quantity into separate cultures, at least one of the cultures being a control,

(c) exposing the cells within each culture that is not a control to one of said antigens, wherein the cells in each culture exhibit a biological activity,

(d) exposing the cells within each control culture to an antigen that was not included in step (a), wherein said cells exhibit a threshold biological activity,

(e) comparing the magnitude of each biological activity to the threshold biological activity, and

(f) identifying cells as responders that have biological activity that exceeds the threshold biological activity.

[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

[0012] Skilled artisans recognize that in specific examples 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.

Tissue Sarcoma, Squamous Cell Carcinoma, Squamous Neck Cancer, Stomach (Gastric) Cancer, Cutaneous T-Cell Lymphoma, Testicular Cancer, Throat Cancer, Thymoma, Thymic Carcinoma, Thyroid Cancer, Thyroid Tumors, Transitional Cell Cancer of the Renal Pelvis and Ureter, Urethral Cancer, Uterine Cancer, Endometrial, Uterine Sarcoma, Vaginal Cancer, Vascular Tumors, Vulvar Cancer, Waldenström Macroglobulinemia (Non-Hodgkin Lymphoma) and Wilms Tumor.

[0020] Other and further objects, features, and advantages would be apparent and eventually more readily understood by reading the following specification and be reference to the accompanying drawings forming a part thereof, or any examples of the presently preferred examples of the disclosure given for the purpose of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] FIG. 1 illustrates an example of immunogenic antigen identification for an exemplary Virus X including exposing PBMCs to multiple antigens followed by re-challenge and determination of a threshold and identification of responders and non-responders.

[0022] FIG. 2 provides an example of a mathematical formula for ordering the immunogenicity of each antigen in FIG. 1 and identification of an antigen hierarchy.

[0023] FIG. 3 shows one example of selection of specific antigens based on the antigen hierarchy in FIG. 2.

[0024] FIG. 4 shows one example of selection of specific antigens based on the antigen hierarchy in FIG. 2.

[0025] FIG. 5 shows one example of selection of specific antigens based on the antigen hierarchy in FIG. 2.

[0026] FIG. 6 illustrates an example of immunogenic antigen identification for an exemplary Bacteria Y including exposing PBMCs to multiple antigens followed by re-challenge and determination of a threshold and identification of responders and non-responders.

[0027] FIG. 7 provides an example of a mathematical formula for ordering the immunogenicity of each antigen in FIG. 6 and identification of an antigen hierarchy.

CLAIMS

What is claimed is:

1. A method of establishing a hierarchy of immunogenicity of antigens for a pathogen or cancer, comprising the steps of:

(a) exposing peripheral blood mononuclear cells (PBMCs) obtained from an individual to two or more antigens from a pathogen or cancer in an *in vitro* culture under conditions to allow T cells in the PBMCs that recognize the antigens to expand in quantity to produce expanded T cells,

(b) splitting the expanded T cells into a plurality of separate cultures and adding only one of said antigens into each of the separate cultures;

(c) measuring the magnitude of response of a biological activity that results from the addition of each respective antigen to each of the separate cultures,

(d) comparing the magnitude of response of the biological activity from each of the separate cultures to a threshold value to discriminate expanded T cell cultures that are responders from T cell cultures that are non-responders, wherein the threshold value is established by challenging the expanded T cells with an antigen that was not included in step (a);

identifying each of the separate expanded T cell cultures as responders to the antigen added in step (c) if the biological activity exceeds the threshold and as non-responders to the antigen if the biological activity does not exceed the threshold,

performing the same method using PBMC samples obtained from multiple individuals;

for each antigen, determining the frequency, relative to the number of individuals evaluated, that the expanded T cell cultures are responders and non-responders; and

establishing a hierarchy of immunodominance of the antigens based on a mathematical formula:

$$\frac{(\% \text{ of responders} + 1) \times \text{Magnitude of Biological Response}}{(\% \text{ of non-responders} + 1)}.$$

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 claim 2, wherein the virus is a respiratory virus, optionally wherein the respiratory virus is a parainfluenza virus or metapneumavirus, further optionally wherein the parainfluenza virus is type III or the metapneumavirus is human metapneumavirus.

5. The method of any one of claims 1-4, wherein the method provides information that is utilized in the determination of an immunotherapy for an individual that has been exposed to the pathogen, is at risk for exposure to the pathogen, is susceptible to the pathogen, has disease from the pathogen, is at risk for the cancer, or has the cancer.

6. The method of claim 5, wherein the immunotherapy comprises a vaccine, immunogenic composition, monoclonal antibody, bi-specific T-cell engager (BiTE), oncolytic virus, adoptive T cell transfer, or a combination thereof, optionally wherein the immunotherapy targets more than one antigen on the same cell.

7. A method of obtaining information for determining the immunogenicity of antigens for a pathogen or cancer, comprising the steps of:

(a) exposing T cells from an individual *in vitro* to more than one antigen from a pathogen or cancer under conditions to allow T cells that recognize the antigens to expand in quantity,

(b) splitting the T cells that expanded in quantity into separate cultures, at least one of the cultures being a control,

(c) exposing the cells within each culture that is not a control to one of said antigens, wherein the cells in each culture exhibit a biological activity,

(d) exposing the cells within each control culture to an antigen that was not included in step (a), wherein said cells exhibit a threshold biological activity,

(e) comparing the magnitude of each biological activity to the threshold biological activity, and

(f) identifying cells as responders that have biological activity that exceeds the threshold biological activity.

8. The method of claim 7, wherein the steps are repeated with cells from one or more different individuals.

9. The method of claim 8, wherein a database is generated comprising information on the total number of responders and the total number of non-responders.