human peripheral blood leukocytes (PBLs) together with antigen directly into the spleens of immunocompromised mice. (See Depraetere *et al.*, (2001) J Immunology 166:2929-2936; International Application Publication NO. WO 1999/60846.)

Despite these advances in antibody identification techniques, a need exists for methods for enrichment of antigen-specific plasmablasts useful for the identification of rare monoclonal antibodies, including monoclonal antibodies which recognize more than one antigen. The present inventors have developed an *in vivo* plasmablast enrichment technique effective for identifying antigen-specific plasmablasts. Described herein are *in vivo* antigen-driven plasmablast enrichment techniques which, when used in combination with antigen-specific cell sorting, allow for efficient and high frequency identification of rare and functional monoclonal antibodies without the need for plasma cell propagation or generation of phage-display libraries. The methods provided by the present invention are particularly useful for identifying rare antibodies. The methods provided by the present invention are also particularly useful for identifying an antibody (*e.g.*, a monoclonal antibody) having specificity to more than one antigen.

SUMMARY ~~OF THE INVENTION~~

The present ~~invention~~ disclosure provides, in part, methods for enriching antigen-specific plasmablasts.

In ~~some~~ one aspect~~s~~, ~~the present invention provides~~there is provided a method~~s~~ for enriching antigen-specific plasmablasts, the method comprising obtaining peripheral blood mononuclear cells (PBMCs) from a donor, contacting the PBMCs with at least one antigen to obtain a PBMC/antigen pre-mix, transferring the PBMCs into the spleen of an immunocompromised animal, and isolating the plasmablasts from the spleen of the immunocompromised animal, thereby enriching antigen-specific plasmablasts. In some embodiments, the additional step of washing the PBMCs to remove excess or unbound antigen after contacting the PBMCs with the antigen and prior to transferring the PBMCs into the spleen of the immunocompromised animal is performed. In some embodiments, the additional step of antigen-specific single-cell sorting of the plasmablasts is performed. In other embodiments, the additional step of immunoglobulin cloning from the isolated plasmablasts is performed.

In some ~~aspects~~embodiments, the present ~~invention~~disclosure provides methods for enriching antigen-specific plasmablasts, the method comprising obtaining peripheral blood mononuclear cells (PBMCs)

from a donor, contacting the PBMCs with at least one antigen, washing the PBMCs to remove excess or unbound antigen, transferring the PBMCs into the spleen of an immunocompromised animal, and isolating the plasmablasts from the spleen of the immunocompromised animal, thereby enriching antigen-specific plasmablasts. In some embodiments, the additional step of antigen-specific single-cell sorting of the plasmablasts is performed. In other embodiments, the additional step of immunoglobulin cloning from the isolated plasmablasts is performed.

In some ~~aspects~~embodiments, the PBMCs are obtained from a donor previously exposed to an antigen. Accordingly, in some ~~aspects~~embodiments, the present disclosure~~invention~~ provides methods for enriching antigen-specific plasmablasts, the method comprising obtaining peripheral blood mononuclear cells (PBMCs) from a donor previously exposed to an antigen, contacting the PBMCs with the antigen or a fragment or portion thereof, transferring the PBMCs into the spleen of an immunocompromised animal, and isolating the plasmablasts from the spleen of the immunocompromised animal, thereby enriching antigen-specific plasmablasts. In some embodiments, the additional step of washing the PBMCs to remove excess or unbound antigen after contacting the PBMCs with the antigen and prior to transferring the PBMCs into the spleen of the immunocompromised animal is performed. In some embodiments, the additional step of antigen-specific single-cell sorting of the plasmablasts is performed. In other embodiments, the additional step of immunoglobulin cloning from the isolated plasmablasts is performed.

In some ~~aspects~~embodiments, the present disclosure~~invention~~ provides methods for identifying or isolating an antibody, the method comprising obtaining peripheral blood mononuclear cells (PBMCs) from a donor, contacting the PBMCs with at least one antigen, transferring the PBMCs into the spleen of an immunocompromised animal, isolating the plasmablasts from the spleen of the immunocompromised animal, and sorting the isolated antigen-specific plasmablasts, thereby identifying or isolating an antibody. In some embodiments, the additional step of washing the PBMCs to remove excess or unbound antigen after contacting the PBMCs with the antigen and prior to transferring the PBMCs into the spleen of the immunocompromised animal is performed. In some embodiments, the additional step of immunoglobulin cloning from the isolated plasmablasts is performed.

In some ~~aspects~~ embodiments of the present methods, the antigen used in the step of contacting the PBMCs with antigen is the same antigen (or a fragment or portion thereof) as the antigen to which the

donor was previously exposed. In other embodiments, the antigen used in the step of contacting the PBMCs with antigen is a different antigen from the antigen to which the donor was previously exposed (*e.g.*, the antigen is a variant, a subtype, an isotype, or a homolog of the antigen to which the donor was previously exposed).

In ~~some~~ one aspects, ~~the present invention provides~~there is provided a methods for obtaining a plasmablast population enriched for plasmablasts having specificity to an antigen, the method comprising obtaining peripheral blood mononuclear cells (PBMCs) from a donor, contacting the PBMCs with the antigen or a fragment or portion thereof to obtain a PBMC/antigen pre-mix, transferring the PBMCs into the spleen of an immunocompromised animal, and isolating the plasmablasts from the spleen of the immunocompromised animal, thereby obtaining a plasmablast population enriched for plasmablasts having specificity to an antigen. In some embodiments, the additional step of washing the PBMCs to remove excess or unbound antigen after contacting the PBMCs with the antigen and prior to transferring the PBMCs into the spleen of the immunocompromised animal is performed. In some embodiments, the enriched plasmablast population contains at least one antigen-specific plasmablast having specificity to the antigen. In some embodiments, the enriched plasmablast population contains at least one antigen-specific antibody-producing plasmablast having specificity to the antigen. In some embodiments, the additional step of antigen-specific single-cell sorting of the plasmablasts is performed. In other embodiments, the additional step of immunoglobulin cloning from the isolated plasmablasts is performed.

In other ~~aspects~~embodiments, the present disclosure~~invention~~ provides methods for obtaining a plasmablast population enriched for plasmablasts having specificity to an antigen, the method comprising obtaining peripheral blood mononuclear cells (PBMCs) from a donor previously exposed to the antigen, contacting the PBMCs with the antigen or a fragment or portion thereof, transferring the PBMCs into the spleen of an immunocompromised animal, and isolating the plasmablasts from the spleen of the immunocompromised animal, thereby obtaining a plasmablast population enriched for plasmablasts having specificity to an antigen. In some embodiments, the additional step of washing the PBMCs to remove excess or unbound antigen after contacting the PBMCs with the antigen and prior to transferring the PBMCs into the spleen of the immunocompromised animal is performed. In some embodiments, the enriched plasmablast population contains at least one antigen-specific plasmablast having specificity to the antigen. In some embodiments, the enriched plasmablast population contains at least one antigen-specific antibody-producing plasmablast having specificity to the antigen. In some

embodiments, the additional step of antigen-specific single-cell sorting of the plasmablasts is performed. In other embodiments, the additional step of immunoglobulin cloning from the isolated plasmablasts is performed.

In one aspect, there is provided a method for identifying a plasmablast capable of producing an antibody having specificity to an antigen, the method comprising obtaining peripheral blood mononuclear cells (PBMCs) from a donor, contacting the PBMCs with at least one antigen to obtain a PBMC/antigen pre-mix, transferring the PBMCs into the spleen of an immunocompromised animal, isolating the plasmablasts from the spleen of the immunocompromised animal, and antigen-specific cell sorting of the isolated plasmablasts, thereby identifying a plasmablast capable of producing an antibody having specificity to an antigen.

In ~~other~~ one aspects, ~~the present invention provides~~there is provided a methods for ~~a~~ identifying a plasmablast capable of producing an antibody having specificity to more than one antigen, the method comprising obtaining peripheral blood mononuclear cells (PBMCs) from a donor, contacting the PBMCs with two or more different antigens to obtain a PBMC/antigen pre-mix, transferring the PBMCs into the spleen of an immunocompromised animal, isolating the plasmablasts from the spleen of the immunocompromised animal, and antigen-specific cell sorting of the isolated plasmablasts, thereby identifying a plasmablast capable of producing an antibody having specificity to an antigen. In some embodiments, the method further comprises washing the PBMCs to remove excess or unbound antigen from the PBMC/antigen pre-mix prior to transferring the PBMCs into the spleen of the immunocompromised. In other embodiments, the method further comprises cloning of the immunoglobulin from the isolated plasmablast.

In some ~~aspects~~embodiments, the PBMCs are obtained from a mammal. In some embodiments, the PBMCs are obtained from a mouse, a rat, a rabbit, or a goat. In other embodiments, the PBMCs are obtained from a human. In other ~~aspects~~embodiments, the PBMCs are obtained from an animal which is a non-mammal.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 sets forth data showing plasmablast differentiation in SCID mice *in vivo*. Human peripheral blood mononuclear cells (PBMCs) prior to transplant (indicated as day 0) or splenocytes obtained from SCID mice on day 4, 7, and 8 (following intrasplenic injection of human PBMCs) were stained with anti-CD19 and anti-CD38 to distinguish B lineage cells. Upper circles, lower circles, and rectangles represent plasmablasts, activated germinal center-like cells, or naïve B cells, respectively. Numbers indicate frequencies of each subset as percent.

Figure 2A shows a representative profile of human donor PBMCs (tetanus toxoid reactive) at day 7 post tetanus vaccination. Figure 2B shows a representative profile of spleen cells (tetanus toxoid reactive) obtained from SCID mice at day 8 after transplantation of human PBMCs obtained from tetanus vaccinated donors. Numbers indicate frequencies of each subset