

METHODS OF ANTIBODY CHARACTERIZATION

[0001] The benefit under 35 U.S.C. §119(e) of U.S. Provisional Application No. 63/420,965, filed October 31, 2022, is hereby claimed, and the disclosure thereof is hereby incorporated by reference herein.

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FIELD

[0002] The present disclosure relates to high-throughput methods of characterizing antibody-target protein interactions using antibody containing supernatant from individually cultured B cells.

BACKGROUND

10 [0003] Therapeutic monoclonal antibody (mAb) discovery typically begins with animal immunization, B cell isolation, and hybridoma formation and culture to generate large panels of mAbs per target. Identifying therapeutic candidates from these large panels involves an assessment of binding affinity, cross-reactivity, epitope binding, blocking activity, and antibody chain compositions. Given the importance of identifying candidate antibodies having the desired
15 functional attributes, hybridoma formation and culture has been considered an essential component of the discovery process to ensure sufficient quantities of antibody are available for a thorough assessment. However, due to the lengthy time involved in hybridoma production, it can take more than 15 weeks to identify initial candidate molecules. With the high cost of developing a therapeutic mAb, the ability to identify quality leads more quickly and inexpensively would
20 provide a significant advantage over current state of the art methods. The present disclosure is directed at overcoming these and other deficiencies in the art.

SUMMARY

[0004] A first example of the present disclosure is directed to a method of characterizing immunogen-binding antibodies from a preparation of B cells. This method comprises providing a
25 preparation of non-immortalized B cells, where B cells of the preparation secrete immunogen-binding antibodies, and culturing the B cells of the preparation individually under conditions effective for the B cells to secrete the immunogen-binding antibodies into culture supernatant. The culture supernatants containing the secreted antibodies are collected from the individually cultured B cells, and each collected supernatant is subjected to two or more different binding assays to
30 characterize the immunogen-binding antibodies from the preparation of B cells.

[0005] Another example of the present disclosure is directed to a method of characterizing binding affinity of antibodies from a preparation of B cells. This method comprises providing a preparation of non-immortalized B cells, where B cells of the preparation secrete immunogen-binding antibodies. The method further involves culturing B cells of the preparation individually under conditions effective for the B cells to secrete immunogen-binding antibodies into culture supernatant. The culture supernatants containing the secreted antibodies are collected from the individually cultured B cells, and each culture supernatant is exposed to increasing concentrations of the immunogen, a fragment of the immunogen, or a homolog of the immunogen. The method further involves detecting association and dissociation between the secreted antibodies of the culture supernatant and the immunogen, fragment thereof, or homolog thereof at each of the increasing concentrations. Binding affinity of the secreted antibodies is characterized based on said detecting.

[0005a] In one aspect, the present disclosure is directed to a method of characterizing binding affinity of antibodies from a preparation of B cells, said method comprising: providing a preparation of non-immortalized B cells, wherein B cells of the preparation secrete immunogen-binding antibodies; culturing B cells of the preparation individually under conditions effective for said B cells to secrete immunogen-binding antibodies into culture supernatant; collecting the culture supernatants containing the secreted antibodies from the individually cultured B cells; exposing each culture supernatant to increasing concentrations of the immunogen, a fragment of the immunogen, or a homolog of the immunogen; detecting association and dissociation between the secreted antibodies of the culture supernatant and the immunogen, fragment thereof, or homolog thereof at each of the increasing concentrations; and characterizing binding affinity of said secreted antibodies based on said detecting.

[0006] Classical methods for identifying antigen-specific antibodies involve harvesting the spleen and/or lymph nodes from immunized animals (*e.g.*, mice), collecting B cells from the harvested tissue, and, due to challenges associated with *ex vivo* culture and survival of B cells, the collected B cells are immortalized. Immortalization is typically achieved via fusing the B cells with immortalized myeloma cells to produce a hybridoma. The immortalized cells are plated into wells and cultured to produce a supernatant rich in antibody that can be used in various assays such as antigen binding, affinity, blocking activity (*e.g.*, receptor-ligand blocking), light chain determination, epitope binning, etc. to select lead antibodies possessing the desired characteristics (see FIG. 1A). Following selection of leads, sequencing is initiated to resolve the sequence of the antibody heavy and light chains required for binding and sequence transfer for recombinant production.

[0007] The primary disadvantage of the hybridoma technology is time. The protocol, excluding immunization, takes ~4-6 months to identify lead candidate antibodies (see Pedrioli, A. and Oxenium, A., “Single B Cell Technologies for Monoclonal Antibody Discovery,” *Trends Immunol.* 42(12): 1143-1158 (2021)). Additionally, the procedure can be low throughput in terms of fusion efficiency and hybridoma formation and some hybridomas are low-yield antibody producers.

[0008] To reduce the time involved and other disadvantages associated with hybridoma technology, advances have been made in defining culture conditions for primary B cells that avoid immortalization and allow for single B cell screening methodologies. Many of these screening approaches involve placing individual B cells in miniaturized wells or chambers, microcapillaries, or water-in-oil droplets, which contain a detectable antigen (either in suspension or immobilized)

CLAIMS

1. A method of characterizing binding affinity of antibodies from a preparation
5 of B cells, said method comprising:
 providing a preparation of non-immortalized B cells, wherein B cells of the
preparation secrete immunogen-binding antibodies;
 culturing B cells of the preparation individually under conditions effective for said
B cells to secrete immunogen-binding antibodies into culture supernatant;
10 collecting the culture supernatants containing the secreted antibodies from the
individually cultured B cells;
 exposing each culture supernatant to increasing concentrations of the immunogen,
a fragment of the immunogen, or a homolog of the immunogen;
 detecting association and dissociation between the secreted antibodies of the culture
15 supernatant and the immunogen, fragment thereof, or homolog thereof at each of the increasing
concentrations; and
 characterizing binding affinity of said secreted antibodies based on said detecting.
2. The method of claim 1 further comprising:
 providing a solid support comprising a plurality of reaction surfaces, wherein each
20 reaction surface comprises a capture reagent immobilized to said surface;
 contacting each of the collected culture supernatants with the solid support under
conditions effective for secreted antibodies from one collected culture supernatant to bind to the
immobilized capture reagent on one reaction surface to form an array of captured antibodies on the
solid support, wherein said exposing, detecting, and characterizing are carried out on the solid
25 support.
3. The method of claim 2 further comprising:
 contacting the array of capture antibodies with a candidate cross-reactive binding
molecule prior to said exposing and
 determining presence or absence of an interaction between the candidate cross-
30 reactive binding molecule and the captured antibodies; and
 identifying binding cross-reactivity of the captured antibodies as a result of said
determining.
4. The method of claim 2 further comprising:

contacting, after said characterizing, the array of capture antibodies with one or more additional binding analytes and

determining presence or absence of an interaction between the one or more additional binding analytes and the captured antibodies to characterize an additional binding
5 characteristic of the captured antibodies.

5. The method of claim 4, wherein the one or more additional binding analytes comprises a binding partner of the immunogen, and blocking activity of the captured antibodies is characterized based on said determining.

6. The method of claim 4, wherein the one or more additional binding analytes
10 is an agent that binds an antibody light chain, and light chain composition of the captured antibodies is characterized based on said determining.

7. The method of claim 2, wherein said detecting and characterizing are carried out using surface plasmon resonance imaging.

8. The method of claim 2, wherein said contacting comprises:
15 flowing each culture supernatant over a reaction surface of the solid support surface,
and
repeating said flowing for at least 15 minutes.

9. The method of claim 8, wherein said repeating is carried out for about 20 minutes to about 40 minutes.

20 10. The method of claim 8, wherein said flowing comprises bidirectional flowing.

11. The method of claim 2, wherein each of the culture supernatants contacted with the reaction surfaces of the solid support comprise a volume of about 150 μ l to about 200 μ l and contain about 20 ng/mL to about 100 ng/mL of secreted antibody .

25 12. The method of claim 2, wherein the solid support comprises 96 to 384 reaction surfaces and said contacting comprises:

contacting each reaction surface with a different culture supernatant, thereby capturing antibodies from 96 to 384 different culture supernatants on the solid support.

30 13. The method of claim 2, wherein the immobilized capture reagent comprises an anti-Fc specific antibody.

14. The method of claim 2, wherein the immobilized capture reagent comprises an anti-light chain specific antibody.

15. The method of claim 1, wherein the preparation of B cells is a preparation of primary B cells.

5 16. The method of claim 1, wherein the preparation of B cells is from a subject immunized with the immunogen.

17. The method of claim 16, wherein the immunized subject is selected from a human, non-human primate, horse, pig, cow, goat, sheep, llama, camel, rabbit, dog, rat, and mouse.

10 18. The method of claim 1, wherein the preparation of B cells is from a subject having an autoimmune disorder.

19. The method of claim 1, wherein the B cells of the preparation produce human antibodies.

20. The method of claim 1, wherein the immunogen-binding antibodies are human antibodies.

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