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# On the use of combinatorial antibody libraries to clone the "fossil record" of an individual's immune response

(passive antibody/AIDS/immunological memory/catalytic antibodies)

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**ABSTRACT** **For about the last century the record of immunological events could only be obtained from serum proteins. We suggest that in the future this will change and the far more detailed nucleic acid record will provide new insights into immunological processes as well as selective access to the complete repertoire.**

Study of the immunological response of man is mainly concerned with the current activity of the immune system. Thus, we are often interested in characterizing the antibody response to a viral infection or in analyzing unusual distributions of immunoglobulins in certain neoplasias and paraproteinemias. However, in a number of situations we may wish to obtain a record, or even the actual products, of immunological events from an earlier time. For instance, in viral diseases where the pathogen may change frequently, the antibody record can be used to map encounters with predecessors of contemporary viruses. As we now begin to contemplate an expanded role for passive immunization in prevention and therapy of disease, these considerations become of even greater significance. In AIDS, cancer, and aging, where immune functions undergo progressive deterioration, it may be advantageous to use passive antibodies that were generated at a time prior to loss of immunological capacity. To accomplish this it will be necessary to use methods that can find, acquire, and amplify such antibodies.

Although it is clear that seroepidemiological studies benefit from the tracing of past encounters with antigen, it could be argued that this is unnecessary for passive antibody therapy, when a contemporary disease process is being combated. All that is required is an effective antibody, and neither the particular individual nor the stage of disease providing it affects the issue, because antibodies, unlike cells, are easily transferred between individuals. Nevertheless, certain individuals may have greater ability than others to combat disease, or important antibody specificities may be unusual, and in both instances we will require special powerful methods for retrieving the products of rare immunological events. More interestingly, the development of antibody defense against disease may not be a concerted process and just because an antibody has disappeared does not imply that it had no role or that later "more mature" antibodies are capable of acting alone. For example, in the defense against virus infection there is a competition between the immune and viral systems, each of which is capable of mutation and selection. A successful outcome for the host requires that the virus be driven to a point where it can no longer evade the host defense. This may require action of a sequence of antibodies such that each move and countermove ends in an immunological checkmate. To recapitulate this successful outcome by passive transfer of immune globulins, it may be necessary to clone many historical members of the antibody cascade, some of which will be stored in memory cells.

As long as we were confined to techniques that used serum products or intact cells, we were essentially restricted to studies of the current antibody capabilities of an individual. To be sure, cell culture techniques together with antigenic stimulation could be used to obtain a glimpse of an individual's immunologic memory, but this is not done without difficulty, and cumbersome additional steps are necessary to amplify and actually obtain any quantities of purified antibody. The development of methods for preparing combinatorial antibody libraries and screening them with phage systems opens the possibility of cloning and recovering most of the antibodies that an individual has ever made (1–9). The current technology is of sufficient power so that the actual size of the library or ability to screen it should not be limiting. We routinely prepare combinatorial libraries of $10^8$ members that, when expressed on the surface of filamentous phage, can be enriched by affinity methods so that in about four steps almost all of the clones are antigen specific (5–7). Thus, the problem reduces to whether one has access to the cells and mRNA that encode the current and past antibody responses. For the current response, there is an abundant source of mRNA in plasma cells, which, in man, largely reside in the bone marrow, from which large combinatorial antibody libraries with defined antigen specificities have been successfully prepared (5).

However, one also needs to recover molecules that exist only in memory cells, and access to mRNA from these cells presents a more formidable problem. This is because the exact location of memory cells is uncertain and the amount of specific mRNA is much less than that in plasma cells. But enough is known to be optimistic; we have some knowledge of the location and lifetime of memory B cells and the markers that can be used for enrichment, as has been recently authoritatively reviewed by Vitetta and her colleagues (10). There appears to be a consensus that B cells, after generation in germinal centers of lymph nodes and spleen, return to the marrow to constitute a pool of long-term memory cells (10). At least some memory cells can last for the lifetime of an individual (11). The current debate about whether continuing presence of low amounts of antigen or mitogenic stimulation is necessary for maintenance of memory (12, 13) is not pertinent since, in either case, we can still clone the antibody genes. As Vitetta points out (10), the main hallmark of memory B cells is the presence on their surface of high-affinity antigen receptors in the form of somatically mutated immunoglobulins. Thus, it should be possible to select memory cells on the basis of surface immunoglobulins, by panning and cell sorting procedures, and to enhance enrichment by combinations of additional markers to discriminate between early and late memory cells (10, 14). Aside from the potential practical advantages of preparing combinatorial libraries

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from memory cells, it will be of interest to compare the nature of the antibodies in various subpopulations of memory cells. For example, it would be interesting to trace the progeny of somatically mutated germ-line gene families through various cellular compartments. An important issue that can be answered only by experimentation concerns the question of whether the levels of mRNA that encode surface immunoglobulins in memory cells are sufficient to allow preparation of libraries by PCR amplification. However, given the demonstrated efficiency of the existing methods (4–7, 14), one expects that this will be the case as long as the excess competing RNA from highly activated plasma cells is removed.

Our own interest in the nature of the "fossil record" of an individual's immune response was aroused when we began to prepare combinatorial antibody libraries against human immunodeficiency virus type 1 antigens from seropositive but symptom-free individuals (5). We found the bone marrow to be a rich source for a vast array of different antibodies to gp120 as well as other viral antigens. Since we did not use any cell enrichment techniques, we presume that most of these antibodies are from plasma cells and thus represent the patients' current antibody capabilities. However, as we begin to characterize these antibodies in preparation for clinical trials on the utility of passive immunization in AIDS, we need to consider whether antibodies made earlier in the host–viral encounter when the individual had more of an upper hand, might be of great value. Similar considerations pertain to the source of antibodies for restoration of immunological capability in aging patients, where the immune system is known to be compromised.

In summary, although the process of passive immunization has long played a role in medicine, we have only exploited a fraction of its potential. As large numbers of human monoclonal antibodies from ongoing responses and from the fossil record become available, it is likely that the true potential of antibodies for therapy and prophylaxis will be realized.

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