ANTIBODY-SPECIFIC IMMUNOREGULATION AND THE IMMUNODEFIciENCY OF AGING*

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One manifestation of the aging process is a gradual decrease in the ability of aged individuals to mount a humoral immune response (1-4). Studies at the cellular level demonstrated that much of this diminished reactivity can be attributed to the environment of the aged animal itself, which limits the responsiveness of the available B cells (3-8).

This laboratory (9) and others (10) have observed that, after immunization with a given antigen, immunoregulatory mechanisms develop that are best evidenced by the suppression of the capacity of primary B cells to respond to the same antigenic determinant. In our experiments, this suppression appears to be specific for idiotypic determinants expressed on the primary B cell receptors, because such suppression obtains only for B cells specific for the immunizing antigen and is not capable of suppressing B cells of murine strains differing in the idiotype-allotype genetic locus (9). Thus, for example, BALB/c mice immunized with 2,4-dinitrophenyl (DNP)-hemocyanin and subsequently used as the adoptive irradiated hosts in B cell transfer experiments suppress the response of primary BALB/c DNP-specific B cells but facilitate the response of primary B cells from B10.D2 or CB20 donors that differ from BALB/c mice in the immunoglobulin heavy chain locus.

Because the effects of this antibody-specific immunoregulation appear to be long-lasting, we reasoned that the antigenic experiences of a lifetime could eventuate in the accumulation of suppressive recognition for a large proportion of an individual's normal primary B cell repertoire. The experiments described in this report test this hypothesis. We have assessed the capacity of the environment or cells of aged individuals, who have never been immunized to the hapten DNP, to suppress the responses of primary DNP-specific B cells derived from young syngeneic or allotype allogeneic donors. The results indicate that aged individuals have indeed accumulated the capacity to suppress the responses of syngeneic primary B cells but not the responses of primary B cells differing in the heavy chain allotype locus.

Materials and Methods

Antigens. The proteins hemocyanin (Hy) and bovine serum albumin, and the antigens DNP10-Hy and DNP10-bovine serum albumin were prepared and characterized as previously described (9).

Animals and Immunization. 24-26-mo-old BALB/c male mice and their 2-mo-old cohorts were obtained from the Charles River Breeding Laboratories, Inc., Wilmington, Mass. 2-mo-old

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B10.D2, DBA/2, and CB20 mice were obtained from the Scripps Breeding Colony. Mice to be used as recipients for cell transfer studies were immunized with 0.1 mg of Hy by intraperitoneal injection in complete Freund's adjuvant, followed 4 wk later by an intraperitoneal injection of 0.1 mg of Hy in saline.

Splenic Focus Fragment Culture System. The splenic fragment culture system was carried out as previously described (9, 11, 12). Two types of irradiated recipients were used for these experiments. In one set of experiments, young or old Hy-primed mice were irradiated 1–2 mo after their last injection of Hy with 1,300 rad and used as recipients of limiting dilutions of spleen cell suspensions, obtained 2-4 h thereafter from 2-3-mo-old donors. In the second protocol, young Hy-primed recipients were irradiated at 600 rad and 2 h later received $4 \times 10^7$ spleen cells from nonimmunized 24-26-mo-old donors or 2-3-mo-old cohorts. 8 h thereafter, these mice were again irradiated at 1,000 rad and 2-4 h later were used as recipients for limiting dilutions of normal spleen cells from 2-3-mo-old donors. 16 h after receiving limiting spleen cell dilutions, recipient animals were killed and splenic fragment cultures were prepared. Cultures were stimulated for 3 d with DNP-Hy at a concentration $10^{-6}$ M for DNP. Culture fluids were changed and collected every 3-4 d thereafter, and those collected on days 10 and 13 were assayed for anti-DNP antibody activity by a radioimmunoassay (9, 12).

Results

Table I presents the results obtained when $3 \times 10^6$ BALB/c or B10.D2 spleen cells were transferred to carrier-primed young or old BALB/c recipients. It can be seen that when BALB/c primary spleen cells were transferred to young carrier-primed irradiated BALB/c recipients, 1.1 monoclonal responses were obtained per $10^4$ B cells. When cells of the same population were transferred to 2-yr-old carrier-primed recipients, only 0.4 monoclonal responses were obtained per $10^6$ B cells. This marked suppression of responsiveness was not seen when cells of B10.132 mice, which differ from BALB/c in the heavy chain allotype locus, were used as the source of primary B cells. Similar results were obtained in separate experiments when either DBA/2 or CB20 mice were compared with BALB/c donors in similarly primed young and old BALB/c recipients.

To determine whether the observed suppression was due to the existence of idiotype-specific suppressor cells present in the environment of aged individuals, a second protocol was used. In this case, young carrier-primed individuals were used as recipients for all B cell populations. Before the injection of the donor B cell population,

### Table I

**Antibody-specific Immunoregulation in Aged Mice**

| Donor (B cells) | Recipient (Hy-primed BALB/c) | Clonal responses/$10^5$ B cells* |
|-----------------|-----------------------------|---------------------------------|
| BALB/c          | 2 mo old†                   |                                  |
|                 | 2 yr old                    |                                  |
| B10.D2§         | 2 mo old                    |                                  |
|                 | 2 yr old                    |                                  |

* $3 \times 10^6$ spleen cells were transferred to each recipient. Data represent a minimum of six recipients for each group. Data are presented as the frequency of positive fragment cultures per $10^5$ B cells present in these cultures calculated on the basis that 40% of the donor spleen cells are B cells and the homing and stimulation efficiency of the splenic focus system is 4% (16). Error bars represent the standard error of the means.
† Age of recipients at time of first priming injection of Hy.
§ The frequency of DNP-specific B10.D2 B cells responsive to DNP-Hy in Hy-primed B10.D2 recipients is $12.8/10^5$ B cells.
these recipients had received $4 \times 10^7$ spleen cells from either young or old syngeneic donors. In Table II, it can be seen that carrier-primed recipients of $4 \times 10^7$ spleen cells of young donors facilitated the responses of syngeneic primary B cells in a normal fashion. However, those that had received $4 \times 10^7$ spleen cells from 2-yr-old mice significantly suppressed the responses of primary syngeneic B cells. Again, the responses of primary B10.1312 B cells were normal in recipients either of young or old BALB/c spleen cells.

Recent experiments by S. K. Pierce and N. Speck (personal communication) have demonstrated that antibody-specific immunoregulation induced by antigenic stimulation can be transferred by purified T cells. To determine whether the suppression displayed by recipients of spleen cells from 2-yr-old mice was also T cell dependent, spleen cell suspensions from 2-yr-old mice were treated with anti-Thy-1.2 antiserum plus complement before transfer (13). The results indicate that >80% of the suppression of responses of syngeneic BALB/c B cells could be eliminated by such treatment, but not by treatment with normal mouse serum plus complement.

**Discussion**

In recent years, the realization that the immune system of an individual has the capacity to recognize its own idiotypic determinants has led investigators to propose and investigate the many effects such recognition could have on repertoire expression and stimulation (9, 10, 14). Indeed, it is now assumed by many that the immune system normally exists in a delicate balance between idiotypic and anti-idiotypic networks comprised of both T and B cells, and that changes in repertoire expression or cellular stimulation are accomplished only by the perturbation of this balance. The phenomenon of antibody-specific immunoregulation that we previously described fits into this general context. However, in young individuals, before overt antigenic stimulation, such a suppression of primary B cell responses does not exist because primary B cells respond at maximum frequency in young recipients primed only with the carrier moiety (9). Because such suppression does not occur before antigen contact, this mechanism may not spontaneously exist and thus may not be determinative in either repertoire establishment or the early stages of B cell stimulation in nonimmunized young individuals. The exception to this conclusion would be that because individuals normally interact with an antigenic environment, even that imposed by maternal influences, immunoregulatory networks could become relevant, particularly
for certain antigens and idiotypes, even relatively early in pre- and post-natal development. Additionally, the immunoregulation of a large proportion of B cells could accumulate in aged individuals. To test this general concept, we have used aged mice, which presumably would have maximized the accumulated affects of normal environmental antigenic experience.

The results indicate that the lack of antibody-specific immunoregulation observed in nonimmune young animals does not hold for nonimmune aged animals. Thus, aged individuals and their splenic cell population have accumulated the capacity to suppress the responses of the majority of hapten-specific syngeneic primary B cells in the absence of overt immunization to that hapten. The finding that this is the case for aged animals confirms that it is not the case for young animals. Thus, for much of the B cell repertoire, a carefully balanced network of idiotypes and anti-idiotypes may not obtain as an inherent element of T and B cell repertoire expression but rather may occur only as the result of the development of anti-idiotypic reactivities as a consequence of induced or accumulated antigen-driven stimulatory events.

The demonstration that antibody-specific immunoregulation does occur as a normal consequence of an individual's encounters with the environment may have important consequences for general considerations of immune responsiveness. It has been shown that the existence of anti-idiotypic reactivity can abort the expression of clonotypes within the B cell repertoire (15). If this is true for spontaneously accumulated anti-idiotypic mechanisms, then the B cell repertoire itself may be tailored by the previous antigenic experience of an individual. It would also seem that at least one contributory factor to the immunological deficiency of aging is a consequence of the fact that the immune system of aged individuals reflects a lifetime of antigenic experience. By the generation and accumulation of immunosuppressive recognition of their own receptor idiotypes, it appears that aged individuals down-regulate their own primary immune responses to much of the antigenic universe.

Summary

Experiments were carried out to assess the role of naturally acquired antibody-specific immunoregulation in the immunodeficiency of aged individuals. It was found that >50% of the primary dinitrophenyl-specific BALB/c B cells did not respond in carrier-primed 2-yr-old BALB/c adoptive hosts as compared with similarly primed younger recipients. Similar suppression was observed in carrier-primed younger BALB/c mice that had received $4 \times 10^7$ spleen cells from 2-yr-old BALB/c mice, as opposed to those that had received $4 \times 10^7$ spleen cells from younger mice. This diminution in responsiveness was noted only for syngeneic BALB/c B cells because B cells of strains differing from BALB/c in the heavy chain allotype-idiotypic locus were not suppressed. These findings indicate that old, but not young, mice had developed the capacity to suppress primary B cells bearing receptors expressing much of the syngeneic antibody repertoire. This suppression may play an important causative role in the relatively poor humoral immune responsiveness of aged individuals.

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