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Anti-Idiotypic Response of BALB/c Mice to a Myeloma Protein of BALB/c Origin[1] (40308)

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It has been demonstrated that a BALB/c myeloma protein with anti-DNP activity (Protein-315) can stimulate anti-idiotypic response in several strains of inbred mice, including the strain from which the plasmacytoma MOPC-315 was originally induced (1, 2). Antibodies produced were found to be specific for the antigen-binding site of Protein-315 (1–3). Tungkanak and Sirisinha (3) also reported that the Fc fragment of Protein-315 was not required for the induction of anti-idiotypic response in BALB/c mice. The anti-idiotypic antibody produced in response to stimulation by the Fab-fragment of Protein-315 was indistinguishable from that produced in response to undigested protein (3). The purpose of the present study was to follow the development of an anti-idiotypic antibody response of BALB/c mice to Protein-315, particularly with regard to the ability of these anti-idiotypic antibodies to compete with the hapten for an antigen-binding site on Protein-315. The results showed that the susceptibility of anti-idiotypic antibody to inhibition by excess hapten (DNP-lysine) depends largely on the immunization procedure used, i.e., the anti-idiotypic antibodies produced following a single booster injection showed a marked increase in the ability to compete with DNP-lysine for the antigen-binding site of Protein-315. Evidence available suggests that this change was associated with an increase in the affinity of the anti-idiotypic antibody produced after a booster injection.

Materials and methods. Antigens. Protein-315 and its peptic product (Fv-315) were prepared and purified as described previously (3). Myeloma sera from BALB/c mice carrying MOPC-315, MOPC-460, MOPC-292, Adj.PC-22A, J504, and S176 tumors were kindly provided by Dr. Herman N. Eisen (Massachusetts Institute of Technology, Boston, MA).

Immunization schedule. BALB/c mice of both sexes used in this study were originally obtained from Jackson Laboratory, Bar Habor, Maine. Adult mice were immunized each time with 200 μg of purified Protein-315 distributed at the same two front footpads and four other sites along the back. The primary course of immunization consisted of 3 weekly injections of immunogen in complete Freund's adjuvant, in incomplete Freund's adjuvant, and in potassium phosphate-buffered saline (PBS) pH 7.2, respectively. The animals were bled from orbital venous plexus one week after the third injection and at weekly intervals thereafter. A single booster injection of 200 μg of immunogen in PBS was given 1 week after the mice that received a full course of primary immunization had been bled 4 times. These animals were bled again during the 4 succeeding weeks. A similar second booster injection was given to some of these mice and the animals were thereafter bled as described. Individual sera from the same group (5–10 mice per group) were pooled and kept frozen until analyzed.

Analysis of anti-idiotypic antibody. Anti-idiotypic antibody to Protein-315 was determined by radioimmunoassay using ^{125}I-labeled Protein-315 or Fv-315 as antigen (3). Pooled sera obtained at weekly intervals were analyzed for their antigen-binding capacity, susceptibility to inhibition by excess hapten, and cross-reactivity with five other myeloma A sera of BALB/c origin exactly as described by Tungkanak and Sirisinha (3).

Results. The antigen-binding capacity of BALB/c antisera, as determined by their ability to react with ^{125}I-labeled antigen (Protein-

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315 or Fv-315), could be detected as early as 1 week after completion of the primary course of immunization with Protein-315 (Fig. 1). Neither the antigen-binding capacity nor the sensitivity to inhibition by excess hapten altered much during the 13 weeks of observation period. There was also no demonstrable change in specificity as the hapten inhibition values obtained when either Protein-315 or Fv-315 was used as antigen in the assay system were similar (Fig. 1).

The antigen-binding capacity of these antisera was enhanced following a single booster injection with 200 μg of Protein-315. As shown in Fig. 2, the quantity of labeled antigen precipitated by 10 μl of antiserum increased from less than 40% to more than 60% one week after boosting. It is more interesting however to find that the ability of these post-boosting sera to compete with excess hapten for the antigen-binding site of Protein-315 increased markedly, i.e., within one week the hapten inhibition value decreased from more than 80% to less than 10%, regardless of the type of antigen used in the assay system. Although the susceptibility to inhibition by hapten gradually increased during the next few weeks, the inhibition value did not quite return to the pre-boosting level. Similar but less obvious changes were observed following a second booster injection.

Selected samples of the pre-boosting (week 4) and post-boosting (weeks 7, 9 and 12) antisera were diluted with pooled normal BALB/c serum and then retested for their susceptibility to inhibition by excess hapten. The results showed that the hapten inhibition values gradually increased as the antisera were being diluted (Table I). The effect of dilution on the hapten inhibition value was independent of the type of the antigen used in the assay system.

Despite a marked change in sensitivity to inhibition by hapten of the antisera obtained

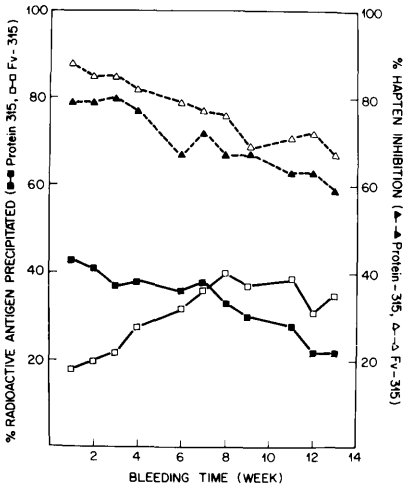

FIG. 1. Antigen-binding capacity and susceptibility to inhibition by excess hapten (750 nanomoles of DNP-lysine) of BALB/c antiserum to Protein-315 from the non-boosting group. Bleeding time represents time after the last injection of the primary course of immunization. Both Protein-315 and Fv 315 were used as antigens in the assay system.

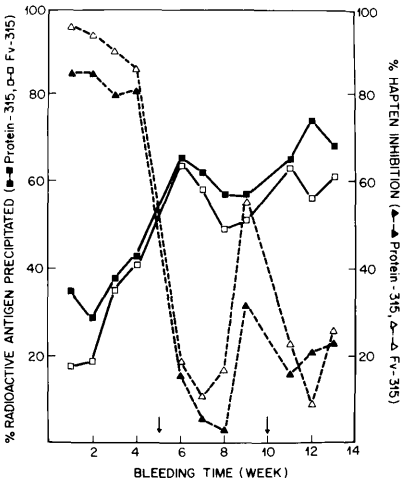

FIG. 2. Antigen-binding capacity and susceptibility to inhibition by excess hapten (750 nanomoles of DNP-lysine) of BALB/c antiserum to Protein-315 from the boosting group. The animals were boosted at weeks 5 and 10 (arrows). See legend to Fig. 1 for other explanations.

TABLE I. EFFECT OF ANTISERUM DILUTION ON HAPTEN INHIBITION[a]

Antiserum	Specimen No. (week)	(%) Maximum hapten inhibition (by 750 nmoles DNP-lys) Undiluted	1:5[b]	1:10	1:20	1:40
Preboosting	4	77	87	92	84	90
Postboosting	7	3	53	65	68	68
	9	36	77	81	82	82
	12	12	55	69	73	73

[a] Excess hapten (750 nanomoles) was mixed with 0.5 μg of ^{125}I-labeled Protein-315 before 10 μl of antiserum (or its dilution) was added. Thereafter the reaction mixture was treated as described in Materials and methods.
[b] Diluted with pooled normal BALB/c serum.

after boosting, the specificity of these antisera remained unchanged. This was evident from the results of a cross-reactivity study using five other myeloma A sera to inhibit the idiotypic reaction. Like the results obtained with the pre-boosting antisera, the myeloma sera from mice carrying MOPC-460, Adj.PC-22A, and S176 tumors failed to inhibit the anti-idiotypic activity of these postboosting antisera while those from MOPC-292 and J504 mice demonstrated slight inhibition (less than 20%).

Discussion. The present observations confirm and extend the original report of Sirisinha and Eisen (1) that under appropriate conditions anti-idiotypic antibody response to a BALB/c myeloma protein with anti-DNP activity can be induced in BALB/c mice. The anti-idiotypic antibody produced is directed largely, if not exclusively, to the antigen-binding site of Protein-315, as evident from the observations that the antisera were highly sensitive to inhibition by excess hapten and they cross-reached only slightly, if any, with five other myeloma A proteins available for testing.

The interesting feature of the anti-idiotypic response is that a single booster injection not only increased the total antigen-binding capacity of these sera but also markedly decreased their susceptibility to inhibition by excess hapten (Fig. 2). The results obtained following a booster injection are markedly different from those obtained after the primary course of immunization. In the non-boosting group, the antisera obtained at weekly intervals throughout the 13 weeks of observation were equally sensitive to inhibition by hapten and their antigen-binding capacity decreased only slightly during this period (Fig. 1).

The change of the hapten inhibition value obtained after boosting was much larger than can be explained on the basis of a quantitative increase of antibody production by these animals. The reduction of the hapten inhibition value must therefore be attributable to changes in other parameters, e.g., affinity and specificity. Athough an increase in affinity of the anti-idiotype produced after a single booster injection is consistent with the general characteristic of a secondary antibody response (5), the possibility that this could also be associated with a shift in specificity cannot be completely ruled out. Circumstantial evidence, however, supports the possibility that a marked reduction in the sensitivity to hapten inhibition of these postboosting sera is more likely associated with an increase in affinity of antibody. Firstly, the hapten inhibition value of the postboosting sera increased when they were diluted prior to testing (Table I). This interpretation is consistent with the explanation of Sher and Cohn (6) who employed the phosphorylcholine system in their study. Secondly, both the binding capacity and hapten inhibition were similar when either Protein-315 or Fv-315 was employed as antigen in the assay system (Fig. 2), suggesting that the reaction is primarily restricted to the Fv region. Lastly, the patterns of cross-reactivity of the pre-boosting and the post-boosting antisera with other myeloma A proteins were indistinguishable from one another (unpublished observation). The possibility that the observed decrease of susceptibility to inhibition by hapten is a laboratory artifact associated with the assay system employed is unlikely as there was a gradual return of these values toward the preboosting level within a few weeks after a booster injection. Likewise, the possibility that there was insufficient labeled antigen in the test system to react with the antibody produced after boostering is also unlikely because under the condition used for the assay of antibody, there was excess antigen left in the supernatant fluid. In addition to this evidence, we may add that a booster injection of antigen under identical condition to other strains of mice (C57BL/6J and A/J) failed to cause any reduction of the hapten inhibition value (unpublished observations). It appears from these observations therefore that the insusceptibility to inhibition by excess hapten of the anti-idiotype produced following a booster injection is more likely associated with an increase in affinity rather than a shift in specificity of these antibodies.

Summary. The anti-idiotypic response of BALB/c mice to myeloma protein of BALB/c origin (purified Protein-315 from a plasmacytoma MOPC-315) was analyzed for its antigen-binding capacity and susceptibility to inhibition by excess hapten (DNP-lysine). The results showed that the anti-idi-

otypic antibody that is sensitive to inhibition by hapten could be detected for at least 3 months after completion of the primary course of immunization. Following a single booster injection, there was an increase of the antigen-binding capacity and the susceptibility of these post-boosting antisera to inhibition by hapten was markedly reduced (from more than 80% to less than 10% under the assay system employed). However, the hapten inhibition value gradually returned toward the preboosting level within a few weeks. The data obtained suggest that the change in the hapten inhibition value after boosting is associated with increased affinity rather than a shift in specificity.

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