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Immunology

Binding to idiotypic determinants of large proportions of thymus-derived lymphocytes in idiotypically suppressed mice

(immune regulation/specificity and stimulation of thymus-derived lymphocytes)

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ABSTRACT **All A/J mice immunized with a conjugate of *p*-azophenylarsonate groups to keyhole limpet hemocyanin produce antibodies against azophenylarsonate, some of which share a crossreactive idiotype. The appearance of the idiotype can be suppressed, without reducing the response against azophenylarsonate, by injecting rabbit anti-idiotypic antibodies prior to immunization. We have now observed that mice suppressed in this way, or by adoptive transfer of leukocytes from other suppressed mice, and then immunized with the hemocyanin–azophenylarsonate conjugate, possess high proportions (up to 14%) of lymphocytes that form rosettes with A/J erythrocytes coated with Fab fragments possessing the idiotype. Idiotypic specificity was demonstrated by various experiments. Most or all of the rosette-forming lymphocytes appear to be thymus-derived lymphocytes (T cells). Treatment of T cells with trypsin eliminated the capacity to form rosettes, which was restored on standing overnight in medium. Thus, the receptors are synthesized by the cells and are not passively adsorbed. Treatment of mice with anti-idiotypic antiserum without antigenic stimulation did not elicit substantial numbers of rosette-forming cells. The requirement for antigen suggests that antigen–idiotype complexes may be a stimulatory agent. A prolonged rest period after immunization of suppressed mice was required for the induction of high percentages of rosette-forming cells. Rosette formation provides a convenient method for studying factors that induce the formation of idiotype-specific T cells.**

Antibodies of a given specificity sometimes show strong intrastrain idiotypic crossreactions, i.e., some of the antibody molecules produced in most or all members of a mouse strain share idiotypic determinants (e.g., refs. 1–4). This is dependent on the antigen and on the strain. The present work is based on the observation that all A/J mice immunized with a conjugate of *p*-azophenylarsonate groups (Ar) to keyhole limpet hemocyanin (KLH) (KLH-Ar) produce antibodies against Ar, of which a substantial proportion share idiotype (2). In contrast, when seven other strains of mice were tested, only one anti-idiotypic (anti-id) antiserum, of three produced against BALB/c anti-Ar antibodies from individual mice, identified *intra*strain idiotypic crossreactions (5). We have proposed that strong idiotypic crossreactivity reflects the product of a germ line gene or of a germ line gene that has undergone a small number of mutations, which occur in every mouse (2). The A/J strain can produce a large spectrum of anti-Ar idiotypes (6), but only one appears with high frequency.

When intrastrain idiotypic crossreactivity exists, one can suppress the crossreactive idiotype (CRI) by administration of anti-id antiserum. This has been done *in vivo* (6–10) and *in vitro* (11), in adult (6–8, 10) and neonatal (6, 9) mice. This suppression does not reduce the amount of anti-Ar antibodies produced by A/J mice (8). Such antibodies possess idiotypes that are not detectable in the anti-Ar antibodies of most other A/J mice, suppressed or nonsuppressed (6, 12).

Eichmann (13) showed that a CRI can be suppressed by adoptive transfer of lymphoid or T cells (thymus-derived lymphocytes) from a suppressed into a mildly irradiated A/J mouse; upon subsequent immunization, the recipient produced antistreptococcal antibodies lacking the characteristic CRI. We have confirmed these findings (12; S-T. Ju and A. Nisonoff, unpublished data) and presented evidence suggesting that the bone-marrow-derived lymphocytes (B cells) of a hyperimmunized suppressed animal may also transfer suppression (14), probably because the immune donor possesses a large excess of B cells, capable of producing anti-Ar antibodies but lacking receptors with the CRI; such cells might compete successfully for antigen.

We have now observed that idiotypically suppressed, hyperimmunized A/J mice develop 6–14% of splenic T cells, with receptors specific for the idiotype of anti-Ar antibodies bearing the CRI. The possible mechanism of induction of idiotype-specific T cells, and its implication with respect to suppression of idiotype, are discussed.

MATERIALS AND METHODS

Materials. A/J mice were obtained from the Jackson Laboratory. Congenic, C.AL-20 mice were the generous gift of Michael Potter; they have the immunoglobulin heavy chain allotype of the AL/N strain on a BALB/c background. Such mice produce anti-Ar antibodies, some of which bear the A/J CRI (15).

Anti-Thy-1.2 antiserum was prepared by injecting thymocytes of C3H/HeJ males into male AKR recipients (16). Serum used as a source of complement was obtained from a 4-week-old rabbit, adsorbed with A/J spleen cells, and frozen.

Preparation of Lymphocytes. The tissue culture medium was RPMI 1640 containing penicillin and streptomycin (GIBCO, Grand Island, NY). "Complete" medium also contained 10% decomplemented, IgG-free, fetal calf serum and 5 μg/ml of gentamycin. Splenic lymphocytes were prepared by teasing spleens in complete medium through stainless steel mesh. Erythrocytes were removed (17) and T-enriched cells were prepared with nylon wool (18). They contained less than 8% B cells, as estimated by fluorescent staining.

Suppression of the CRI of Anti-Ar Antibodies. Male A/J or C.AL-20 mice, 6–8 weeks old, were inoculated intraperitoneally with two 0.5-ml portions of rabbit anti-id antiserum, given 3 days apart. Starting 2 weeks later, the mice were immunized intraperitoneally with 0.5-mg portions of KLH-Ar in complete Freund's adjuvant. The volume-ratio of adjuvant to antigen solution was either 1:1 or 9:1. The latter ratio was used when it was desired to raise an ascitic fluid containing antibodies (19). Antigen was given weekly for 4 or 5 weeks. The sera

Abbreviations: anti-id, anti-idiotypic; Ar, *p*-azophenylarsonate; CFA, complete Freund's adjuvant; CRI, crossreactive idiotype; KLH, keyhole limpet hemocyanin; RBC, red blood cells; T and B cells, thymus-derived and bone-marrow-derived lymphocytes, respectively.

Table 1. Idiotype-specific rosette formation by splenic or T-enriched lymphocytes obtained from mice suppressed for CRI by adoptive transfer, then hyperimmunized*

Red cell coat†	Inhibitor	Mouse no. HIS-28A		Mouse no. HIS-28B	
		Splenic lymphocytes	T-enriched cells‡	Splenic lymphocytes	T-enriched cells‡
Nonspecific Fab	None	0.4	0	0	0
Fab of A/J anti-Ar antibodies	None	8.4	14.0	11.7	10.3
	A/J anti-Ar serum, 0.1 μl§	1.3	0	0	0
	A/J anti-Ar serum, 5 μl	1.2	0.7	0.6	0.2
	Normal A/J serum, 5 μl	7.4	9.3	9.8	9.5
	A/J HIS serum, 5 μl¶	11.5	13.5	7.4	9.5
	$F(ab')_2$ of rabbit anti-id, 100 μg	0.4	0	0.7	0
	$F(ab')_2$ of rabbit anti-MIgG, 100 μg	9.2	9.7	6.9	8.2

* Details of the adoptive transfer of ascitic cells and hyperimmunization are in the *text*. Each recipient produced a high titer of anti-Ar antibodies lacking detectable CRI. Mice were killed 10 weeks after the last inoculation of KLH-Ar. Values are % rosette-forming cells.

† No rosettes were formed when uncoated RBC were used in the tests.

‡ Obtained by elution from nylon wool. T cells (4%) and spleen cells (37%) were stained by fluorescent antiglobulin (indirect method).

§ Containing 1.5 mg of anti-Ar antibody per ml with CRI.

¶ Containing 0.7 mg of anti-Ar antibody per ml lacking CRI (from a suppressed, hyperimmunized A/J mouse).

or ascites of immunized mice were titered for anti-Ar antibodies and CRI. Mice were used that had produced a high titer of anti-Ar antibodies lacking the CRI.

Adoptive transfer of the suppression of idiotype with leukocytes from ascitic fluids (20, 21) was done using mice that had been suppressed for CRI, then repeatedly immunized, using a 9:1 ratio of complete Freund's adjuvant (CFA) to antigen (KLH-Ar) solution. Typically, ascitic fluids contained from 8 $\times 10^6$ to 14 $\times 10^6$ leukocytes per ml; 3 $\times 10^6$ cells were sufficient for suppression of the CRI (*Results*). Recipients were hyperimmunized with four 0.25-mg inoculations of KLH-Ar in CFA over 32 days and bled a week later. Mice with anti-Ar antibodies lacking detectable amounts of CRI were used for subsequent rosetting experiments.

Enumeration of Idiotype-Specific Rosette-Forming Lymphocytes. Fab fragments were conjugated to red blood cells (RBC) with $CrCl_3$ (22). Mixtures contained 1 mg of $CrCl_3$ and 1 mg of protein per ml of packed RBC. After 15 min at 0°, cells were washed repeatedly with complete medium. Each mixture also contained 2 ng of ^{125}I-labeled Fab fragments (about 10,000 cpm) to permit estimation of the degree of incorporation of the Fab fragments; the amount conjugated varied from 30 to 60 ng per 10^6 RBC.

Mixtures were prepared with complete medium containing 2 $\times 10^5$ splenic lymphocytes, or T-enriched lymphocytes, together with 4 $\times 10^6$ A/J RBC coated with Fab fragments of anti-Ar antibodies with the CRI, or with Fab fragments of normal mouse IgG, in 0.2 ml. The mixture was centrifuged at 500 $\times g$ for 5 min at 25° After standing for 30 min at 25° and 30 min at 4°, the pellet was resuspended in the supernatant and examined at a magnification of 400×. A rosette was defined as a lymphocyte with three or more RBC attached. In nearly all cases 250 to 500 lymphocytes were counted.

Treatment of Cells with Trypsin. Lymphocytes (1 $\times 10^7$ in 2 ml) were incubated with 0.2% crystallized trypsin in RPMI 1640 medium at 37° for 30 min in 7% CO_2 and 93% air, washed twice, and tested for rosette-formation.

Other Methods. The following procedures have been described: preparation of rabbit anti-id antibodies (2); iodination of protein (23); and radioimmunoassay for the CRI (2). The assay uses goat anti-rabbit Fc to precipitate complexes of rabbit anti-id antibodies with ^{125}I-labeled mouse anti-Ar. The total content of anti-Ar antibodies was determined by a solid-phase radioimmunoassay (24), using polyvinyl microtiter plates. It was standardized with sera whose anti-Ar content was determined by the quantitative precipitin test (2). All assays were done in duplicate. The purity of Fab fragments (25) was demonstrated by Ouchterlony analysis with rabbit anti-Fab and rabbit anti-Fc antisera.

RESULTS

Idiotype-Specific Rosette Formation by Lymphocytes. The data in Table 1 indicate that splenic or T-enriched splenic lymphocytes from mice that were idiotypically suppressed and then hyperimmunized form rosettes with RBC coated with Fab fragments of anti-Ar antibodies containing the CRI. The two animals used were suppressed by adoptive transfer of ascitic cells from a hyperimmune suppressed mouse, then immunized with KLH-Ar. The two recipient mice were irradiated (200 R) prior to the adoptive transfer, and were then given four intraperitoneal inoculations of 0.5 mg of KLH-Ar in CFA over 7 weeks. Ten weeks later, the mice were killed. The splenic lymphocytes did not form rosettes with RBC coated with Fab fragments of normal A/J IgG (Table 1). However, a very substantial percentage (8.4–14%) formed rosettes when the RBC were coated with Fab fragments of purified anti-Ar antibodies possessing the CRI. Specificity for the CRI was indicated, first, by the failure to form rosettes with RBC coated with nonspecific Fab. Also, serum containing anti-Ar with the CRI was a potent inhibitor of rosette formation. Normal A/J serum and A/J serum with a high titer of anti-Ar antibodies lacking the CRI (from a suppressed mouse) were noninhibitory. Thus, anti-Ar antibodies possessing CRI appear to interact with receptors on T cells and compete successfully for the corresponding receptors on the coated RBC. Since anti-Ar antibodies lacking the CRI were noninhibitory, the possibility that rosette formation is caused by KLH-Ar adsorbed to lymphocytes is excluded. The presence of $F(ab')_2$ fragments of rabbit anti-id antibody inhibited rosette formation, whereas $F(ab')_2$ fragments of rabbit anti-mouse IgG were not inhibitory. The inhibition may be attributed to interaction of anti-id antibodies with determinants on the coated RBC. The rabbit anti-mouse IgG had anti-Fab activity and must have reacted with Fab fragments on the RBC; idiotypic determinants evidently remained exposed.

Inhibition of Rosette Formation by Haptens. The interaction of antihapten antibodies with their specific haptens

Table 2. Time dependence, after immunization of suppressed mice, of the development of idiotype-specific rosette-forming cells (RFC)*

Source of splenic lymphocytes	No. of mice	Weeks after immunization	% RFC, mean and range
Normal A/J	15		0.1 (0–0.5)
Suppressed, hyperimmunized A/J	5	2–4	2.1 (1.8–2.5)
Suppressed, hyperimmunized A/J	12	7–11	9.4 (6.2–15.5)
Nonsuppressed, KLH-Ar immune mice	8	3–20	0.5 (0–2)

The mice were suppressed by adoptive transfer of ascitic cells from a suppressed, hyperimmunized A/J mouse. They were then hyperimmunized and allowed to rest for the period specified before preparation of splenic lymphocytes for tests of rosette-formation with RBC coated with Fab fragments of anti-Ar antibodies possessing the CRI.

* Evidence for idiotypic specificity of the rosettes is discussed in the *text*.

frequently inhibits the binding to anti-id antibodies (26–30). The effect of haptens on rosette formation was therefore investigated.

The splenic lymphocytes of one suppressed, hyperimmunized mouse, HIS-28C, and the T cells of mouse HIS-28D were used; in the absence of inhibitor 13.2 and 9.2%, respectively, of the lymphocytes formed idiotype-specific rosettes. Specificity was proven by the tests indicated in Table 1. The hapten, (*p*-azobenzenearsonic acid)-*N*-acetyl-L-tyrosine, caused almost complete inhibition at a concentration of 0.1 mM. This compound is the largest tested and should closely resemble side chains in the immunogen. *p*-Aminophenylarsonate (1 mM) caused 70% inhibition in one system and 76% inhibition in the other. *p*-Hydroxyphenylarsonate and phenylarsonate and the unrelated *p*-hydroxybenzoate and *p*-aminobenzoate were essentially noninhibitory. The failure of two phenylarsonate derivatives to inhibit may reflect a low affinity or steric factors (26). Measurement of trypan blue exclusion indicated that the highest concentration of hapten used was not cytotoxic.

Effect of a Rest Period, after Immunization of Suppressed Mice, on Rosette Formation. The data in Table 2 include a larger group of mice and indicate the importance of the rest period used. Mice were suppressed by adoptive transfer of ascitic cells from a hyperimmune suppressed mouse, and then immunized. When 2–4 weeks were allowed to elapse after the last injection of KLH-Ar, an average of 2.1% of the lymphocytes formed idiotype-specific rosettes. This value increased to 9.4% in a group tested 7–11 weeks after the last injection. All 17 mice produced significant numbers of rosettes. Evidence that rosette formation was idiotype-specific was obtained by the tests described in Table 1. In all instances, anti-Ar serum containing the CRI was completely inhibitory, whereas normal A/J serum or anti-Ar serum from a hyperimmune, suppressed mouse had no significant effect.

Nonimmunized A/J mice and nonsuppressed mice immunized with KLH-Ar failed to produce rosette-forming splenic lymphocytes (Table 2). The latter mice were tested 3–20 weeks after the last injection.

Idiotype-Specific Rosette Formation by Lymphocytes from Mice Suppressed by Direct Challenge with Anti-id Antiserum, then Hyperimmunized. The above experiments used mice suppressed by the adoptive transfer of leukocytes from other suppressed mice which, in turn, had been suppressed with anti-id antiserum and immunized. Table 3 shows that idiotype-specific rosettes can also be obtained from mice suppressed with anti-id antiserum and then immunized. The table also presents data obtained with congenic C.AL-20 mice that produce the CRI (15). An additional five A/J mice were tested, with results virtually identical to those shown in the table.

Table 3. Idiotype-specific rosette formation by splenic T cells from A/J or C.AL-20 mice suppressed with anti-id antiserum*

RBC† coat (Fab)	Inhibitor	A/J mouse no. S1	S2	S3	C.AL-20 mouse no. CS1	CS2
Normal A/J IgG	None	0.7	0.3	0.2	0	0.5
Specifically purified anti-Ar antibody	None	12.0	10.0	6.3	6.3	3.2
	Normal A/J serum, 5 μl	11.0	8.7	5.3	8.4	3.3
	A/J anti-Ar serum, 0.1 μl‡	1.6	0.8	1.1	1.2	0
	A/J anti-Ar serum, 5 μl	0.8	0.7	0.6	n.d.	n.d.
	"Suppressed" anti-Ar serum, 5 μl§	9.9	10.0	5.4	8.2	2.1

n.d., not determined.

* Mice were suppressed with two 0.5-ml injections of rabbit anti-id antiserum given 3 days apart. Starting 2 weeks later, they were inoculated five times with 0.5 mg of KLH-Ar in CFA over a 6-week period. Cells were taken 10 or 13 weeks after the last injection of the A/J or C.AL-20 mice, respectively.

† RBC were from the same strain that provided the lymphocytes.

‡ Containing CRI; anti-Ar antibody concentration, 1.7 mg/ml.

§ From a suppressed, hyperimmune mouse not producing CRI; anti-Ar antibody concentration, 0.7 mg/ml.

The methods used are indicated in a footnote of Table 3. The cells were taken 10 and 13 weeks, respectively, after the last injection of the A/J or C.AL-20 mice. The percentages of rosette-forming cells are comparable to those observed after adoptive transfer (Tables 1 and 2), except that a relatively low value was obtained with one of the two C.AL-20 mice. It is of interest that most properties of the T cells of the C.AL-20 strain might be expected to correspond to those of BALB/c rather than A/J mice; further investigation may be warranted.

Requirement for Antigenic Challenge. The data in Table 4 indicate that treatment with anti-id antiserum, without subsequent immunization, yields a much smaller number of idiotype-specific lymphocytes. No rosettes were detected after 8 weeks, and a relatively small percentage was present in three of five mice after 14 weeks. The small percentages are probably significant since the rosettes were inhibited by serum containing the CRI, and were not detected when RBC were coated with nonspecific Fab.

Effect of Exposure of Lymphocytes to Trypsin on Rosette-Forming Capacity. To determine whether rosette formation might be due to passive adsorption of anti-id antibodies onto lymphocytes, idiotype-specific, T-enriched splenic lymphocytes of mouse HIS-5A were treated with trypsin (Table 5). Brief exposure to the enzyme eliminated the capacity to form rosettes. After cells were washed and had stood for 24 hr in complete medium, the percentage of cells that formed rosettes

Table 4. Percentage of idiotype-specific rosette-forming splenic lymphocytes in mice receiving anti-id antiserum without subsequent immunization*

Weeks after administration of anti-id	No. of mice in group	% rosette-forming cells
2	3	< 0.5
4	3	< 0.5
8	6	< 0.5
14	5	0, 0, 0.9, 1.1, 1.3†

Each mouse received two 0.5-ml portions of rabbit anti-id antiserum 3 days apart. Rosettes were prepared using A/J RBC coated with Fab fragments of anti-Ar antibodies.

* Evidence for idiotypic specificity of the rosettes is discussed in the *text*.

† Values for the five individual mice.

was again comparable to that of the untreated cells. This experiment was repeated with T cells of another suppressed, hyperimmunized mouse, HIS-5B, with very similar results (data not shown). Thus, the receptors on T lymphocytes responsible for idiotype-specific rosette formation are synthesized endogenously. The data provide further evidence that rosette formation is not due to the interaction of passively adsorbed KLH-Ar with the coated RBC.

Sensitivity of Rosette-Forming Lymphocytes to Anti-Thy-1.2 Antiserum. These experiments were done both with splenic and T-enriched splenic lymphocytes of mouse HIS-28E (Table 1) that had been suppressed by adoptive transfer, hyperimmunized, and allowed to rest for 11 weeks; 8.4% of the splenic lymphocytes and 14.0% of T-enriched lymphocytes formed idiotype-specific rosettes. When the cells were first treated with anti-Thy-1.2 serum or complement, there was little effect on rosette-formation. A mixture of the two reagents caused complete elimination of the rosette-forming cells from either population of cells.

Other Observations. The presence of sodium azide (0.5 mg/ml) completely inhibited rosette formation. The RBC in rosettes tended to cluster in one region of the lymphocyte surface.

DISCUSSION

It is evident that lymphoid cells from an idiotypically suppressed, hyperimmunized animal may possess a high percentage of splenic lymphocytes capable of forming rosettes with autologous RBC coated with Fab fragments bearing the idiotype. Idiotypic specificity was proved by various experiments.

The inability to inhibit rosettes with anti-mouse IgG (containing anti-Fab) suggests either that the T-cell receptor is not an immunoglobulin or that only the region of the binding site is exposed to the external environment. Antigen-specific T-cell factors are also not affected by anti-immunoglobulin antibodies (31–33).

That receptors on T cells responsible for rosette formation are synthesized by the cells and not passively adsorbed was shown by two experiments in which rosette formation was eliminated by brief exposure to trypsin and regenerated on standing in medium. This also indicates that the rosettes are not formed by the interaction of KLH-Ar, adsorbed on the T cells, with Fab fragments of anti-Ar on the RBC. The latter possibility was also ruled out by the failure of anti-Ar antibodies lacking the CRI to inhibit rosette formation.

Table 5. Loss and regeneration of capacity to form idiotype-specific rosettes after treatment of T-enriched splenic lymphocytes with trypsin*

RBC coat (Fab)	Inhibitor	Incubation, hr†			
		No trypsin		Trypsin	
		0	24	0	24
Normal A/J IgG	None	0	0	0	0
Specifically purified anti-Ar antibody‡	None	11.3	11.3	0	10.1
	Normal A/J serum, 5 μl	8.7	9.7	0	10.4
	Anti-Ar serum, 1 μl§	0.4	0	0	0
	Anti-Ar serum, 5 μl (suppressed)¶	9.1	9.8	0	10.3
	$F(ab')_2$ of anti-id‖	0	0	0	0

* As described in the *text*. Lymphocytes were prepared from a suppressed, hyperimmunized mouse, HIS-5A, 8 weeks after the last injection of KLH-Ar.

† Prior to the test for rosette formation. The cells were incubated in medium.

‡ Containing CRI (nonsuppressed).

§ Containing 1.7 mg of anti-Ar antibody (nonsuppressed) per ml.

¶ From a suppressed, immunized A/J mouse; anti-Ar content, 0.7 mg/ml.

‖ From IgG of rabbit anti-id antiserum; 100 μg added to the RBC.

Idiotype-specific lymphocytes could be obtained from mice that were suppressed directly by anti-id antiserum or indirectly by adoptive transfer of leukocytes from suppressed mice. After challenge with anti-id antiserum, the development of substantial numbers of rosette-forming lymphocytes required challenge by antigen, although a small percentage of idiotype-specific rosettes was detected after 14 weeks without antigenic stimulation. (The method is probably not useful for detection of rosette-forming lymphocytes at a concentration below 0.5%.)

A rest period was required for maximal development of idiotype-specific lymphocytes in suppressed, hyperimmunized mice; the percentages were much greater 8–14 weeks after the last inoculation of antigen than after 2–4 weeks. This may reflect a gradual accumulation of idiotype-specific lymphocytes or, conceivably, the clearance of inhibitory antigen-antibody complexes.

Treatment of splenic lymphocytes with anti-Thy-1.2 antiserum and complement eliminated the capacity to form rosettes. However, in one mouse, the percentages of T-enriched and splenic cells that formed rosettes were about the same. This finding might be reconciled with the view that the rosette-forming cells are mainly T cells, if idiotype-specific T cells, like suppressor cells (34), are preferentially retained on nylon wool or if damage to the cells occurs during the procedure.

The importance of antigenic stimulation suggests that this is a critical factor in inducing such large proportions of idi type-specific cells. Since the cells are specific for idiotype, not antigen, it seems likely that the idiotype is the stimulatory agent, despite the fact that the mice were not producing detectable amounts of the CRI. It seems possible that small amounts of antibodies with CRI are synthesized, which form complexes with the large excess of antigen present, and that the complexes are stimulatory. Eichmann has suggested that such complexes stimulate suppressor T cells (13).

Although the continual presence of serum containing the CRI inhibited rosette formation, exposure of T cells for 30 min to the serum, followed by washing, had no effect. This suggests that the T cells have a low affinity for the idiotype on anti-Ar antibodies in solution. When rosettes are formed, multivalent attachments, with consequent high avidity, may occur. Consistent with this possibility is the fact that free haptens, capable of inhibiting rosette formation, were unable to dissociate rosettes once formed. The removal of the inhibitor by washing may be related to the observation that helper T cells cannot be depleted by passage through a column of adsorbent coated with the specific antigen (35).

Idiotype-specific rosettes were not found in immunized, nonsuppressed animals. However, small percentages of T cells (<0.5%), which might be significant in regulation, would not be detected by the rather insensitive rosetting technique. The data are thus not necessarily germane to this question.

It is apparent that the rosette-forming cells might interact with B cells having receptors with the appropriate idiotype. It thus seems possible that suppression of idiotype is mediated by direct interaction between T and B cells in this system. Such an interpretation must be made with caution in view of the fact that suppressor T cells usually interact with other T cells (36) and the report of Herzenberg *et al.* (37) that allotype-specific suppression of B cells is mediated by the suppression of a subpopulation of helper T cells; one might have predicted that B cells were the target. The mechanism of suppression therefore remains to be elucidated.

Experiments to be reported elsewhere (F. L. Owen, S-T. Ju, and A. Nisonoff, unpublished data) indicate that the idiotype-specific, rosette-forming T cell population includes the cells responsible for suppression of the CRI. That T cells can act as helper cells by combining with idiotypic determinants on a myeloma protein is indicated by the work of Janeway *et al.* (38).

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