PASSIVE TRANSFER OF THE IDIOTYPICALLY
SUPPRESSED STATE BY SERUM FROM SUPPRESSED
MICE AND TRANSFER OF SUPPRESSION
FROM MOTHERS TO OFFSPRING*

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An idiotype, designated CRI_A, associated with a large proportion of anti-p-azo-phenylarsonate (anti-Ar)[1] antibodies of A/J mice, can be suppresssed in adult mice by administration of rabbit anti-idiotypic (anti-Id) antibody (1, 2) or a monoclonal mouse anti-Id antibody (3) before immunization with an Ar-protein conjugate. When such suppressed mice are hyperimmunized, they produce high titers of anti-Ar antibodies that lack CRI_A. After a rest period of 8–12 wk, the hyperimmunized, suppressed (HIS) mice develop high concentrations of suppressor T cells with anti-Id receptors (4, 5). The T cells of HIS mice that have been allowed to rest for a shorter period of time elaborate molecules with idiotypic or anti-Id receptors, either of which can suppress the CRI_A component of the humoral anti-Ar response (6). In the present report, we show that when T cells from HIS mice are transferred into female mice just before mating, their offspring, when immunized shortly after birth, are unable to produce CRI_A, although their capacity to make anti-Ar antibodies is unimpaired. In addition, the sera of HIS mice contain soluble factor(s) which, when transferred into adult mice, selectively suppress the formation of anti-Ar antibodies expressing CRI_A. When HIS serum is inoculated into pregnant females 5–7 d before parturition, or directly into neonatal mice, it suppresses production of the idiotype in newborn mice. The results indicate that the suppressive factor is not an immunoglobulin (Ig).

Materials and Methods

Mice. Strain A/J mice were obtained from The Jackson Laboratory, Bar Harbor, ME.

Preparation of Anti-Id Antibodies. Anti-Ar antibodies were specifically purified from ascitic fluids (7), induced in A/J mice that had been hyperimmunized with keyhole limpet hemocyanin (KLH)-Ar. The antibodies were purified by affinity chromatography on Sepharose 4B (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ) to which bovine IgG-Ar was conjugated; they were eluted with 0.5 M p-aminophenylarsonate, and the hapten was subsequently removed by exhaustive dialysis (8). Anti-Id antibodies were prepared in rabbits by

repeated inoculation of specifically purified A/J anti-Ar antibodies in complete Freund's adjuvant (CFA) (1). Anti-Id antisera were exhaustively adsorbed on columns of Sepharose 4B to which nonspecific crude A/J globulins were conjugated. The idiotypic specificity of adsorbed antibodies was demonstrated by appropriate inhibition tests in radioimmunoassays.

Assays. Serum anti-Ar antibodies were quantified according to Klinman et al. (9). Polyvinylchloride microtiter plates (Fisher Scientific Co., Pittsburgh, PA) were coated with bovine serum albumin-Ar. Samples containing mouse anti-Ar antibodies were added to the wells; this was followed, after washing, by the addition of ^{125}I-labeled specifically purified rabbit anti-mouse Fab. Mouse sera of known anti-Ar content and anti-Ar hybridoma products were used as standards. Quantitative assays for the CRI$_A$ content of A/J anti-Ar antibodies were carried out by inhibition in a radioimmunoassay as previously described (8, 10); 10 ng of ^{125}I-labeled purified A/J anti-Ar antibody was used as the ligand, and immune complexes were precipitated with excess goat anti-rabbit Fc. Each assay mixture also contained 5 μl of nonimmune A/J serum to absorb any trace of residual antibody activity directed to nonidiotypic determinants. ^{22}NaCl was also present in each mixture; measurement of the content of ^{22}Na in supernatants and precipitates eliminates the necessity for washing precipitates.

Quantitative assays for anti-CRI$_A$ in mouse serum were carried out by coating the wells of polyvinylchloride microtiter plates with CRI$_A$-positive A/J anti-Ar antibodies, saturating with 2% horse serum, and adding the unknown, followed by 10 ng of ^{125}I-labeled monoclonal anti-CRI$_A$. A standard solution containing 50 ng/ml of unlabeled monoclonal anti-CRI$_A$ caused more than 50% inhibition in the assay.

HIS Mice; CRI$_A$-negative Anti-Ar. A/J mice, generally 8–10 wk old, were suppressed with respect to CRI$_A$ as described previously (1, 4). In brief, mice were given two intraperitoneal injections, 3 d apart, of rabbit anti-Id antiserum (100 μg idiotype-binding capacity per inoculation). Starting 2 wk later the mice were hyperimmunized with three intraperitoneal injections, at 2-wk intervals, of 250 μg KLH-Ar in CFA. Only mice that produced undetectable titers of CRI$_A$ were used as a source of suppressor spleen cells or soluble suppressor factor. Such mice expressed high serum titers of anti-Ar antibodies, but 25,000 ng of their anti-Ar antibodies failed to cause 50% inhibition in the assay for CRI$_A$.

Cell Fractionation. Single-spleen cell suspensions prepared from HIS mice were exposed to a solution containing 0.15 M NH$_4$Cl, 0.01 M KHCO$_3$, and 0.1 mM ethylenediamine-tetracetate, pH 7.4, for 3 min at 0°C, to lyse erythrocytes (11). The cells were enriched for T cells by the method of Mage et al. (12). In brief, 5 × 10^7 leukocytes in Dulbecco's modified Eagle's medium, containing 1% heat-inactivated fetal calf serum, were incubated in a 100-mm polystyrene petri dish that had been precoated with specifically purified rabbit anti-mouse Fab (1 mg/ml). The average recovery of cells from individual plates was 34% of the total. More than 95% of the recovered cells were killed by treatment with monoclonal anti-Thy-1.2 plus rabbit complement (C) (Low-Tox complement; Cedarlane Laboratories, Hicksville, NY). A population enriched for B cells was obtained by removing the cells adherent to the anti-Fab-coated plate by adding medium and agitating with a rubber policeman at room temperature. The recovered cells were incubated with anti-Thy-1.2 antiserum and C to kill any residual T cells. Alternatively, enriched B cell populations were prepared by treating the whole spleen cell suspension twice with anti-Thy-1.2 and C. Less than 5% of the B cells enriched by either procedure could be killed by anti-Thy-1.2 plus C.

Other Reagents. Goat anti-rabbit IgG was obtained from Antibodies, Inc., Davis, CA. Rabbit anti-mouse Fab was purified by affinity chromatography on Sepharose 4B conjugated with Fab fragments; 0.1 M glycine-HCl, pH 2.5, was used for elution. The method employing cyanogen bromide was used for conjugating proteins to Sepharose (13).

Results

Table I shows the results of experiments in which splenic leukocytes from HIS donors were transferred to female mice, which were then mated; the mice that were studied became pregnant within 7 d. Both the females and their litters were immunized. Immunization of the female parents was started within 24 h after parturition. They were given 100 μg of KLH-Ar in CFA on days 0 and 14 and bled on day 28.

TABLE I

Expression of CRI$_A$ in Litters of Mothers Given Cells From Hyperimmune, or Idiotypically Suppressed Hyperimmune Mice

Group	Cells transferred*	Number of litters	Mothers (M) or litters (L)	Day 28 sera	
				Anti-Ar titer	Anti-Ar antibody required for 50% inhibition‡
				mg/ml	ng
1	None	1	M	1.7	44
			L	(0.18, 0.14, 0.27, 0.55)	(62, <10, <10, <10)
2	2.5 × 10⁶ hyperimmune T cells§	2	M	2.1, 2.7	71, <10
			L	(0.68, 0.13) (0.21, 0.23, 0.13)	(77, 73) (<10, <10, 26)
3	2.5 × 10⁶ hyperimmune suppressed T cells¶	3	M	1.1, 2.2, 2.2	(>5,000(0),‖ >5,000(9), >5,000(0)
			L	(0.29, 0.20, 0.23)	(>5,000[5], 2600, >5,000[48])
				(0.67, 0.12, 0.97) (0.99, 0.72, 0.22)	(2600, 2800, 2900) (3300, 3900, 2600)
4	1 × 10⁷ hyperimmune suppressed T cells	2	M	2.5, 1.9	>5,000(6), >5,000(3)
			L	(0.63, 0.25)	(>5,000[3], >5,000[0])
				(0.29, 0.13, 0.98)	(>5,000[1], >5,000[0], >5,000[0])
5	5 × 10⁵ hyperimmune suppressed T cells	2	M	1.5, 0.60	<10, <10
			L	(0.82, 0.31) (0.20, 0.33, 0.12, 0.10)	(<10, <10) (<10, <10, <10, <10)
6	2.5 × 10⁶ hyperimmune B cells	2	M	2.0, 5.4	33, <10
			L	(0.26, 0.13, 0.27)	(<10, <10, <10)
				(0.26, 0.22, 0.53, 0.22, 0.37)	(<10, <10, <10, <10, <10)
7	2.5 × 10⁶ hyperimmune suppressed B cells	4	M	3.2, 1.7, 0.16, 0.27	18, 440, 310, <10
			L	(0.021, 0.18, 0.033) (0.93, 0.047, 0.061)	(44, 21, 67) (170, 50, 79)
				(0.61, 0.13, 0.24) (0.30, 0.34, 0.023)	(220, <10, 370) (<10, <10, 27)
8	1 × 10⁷ hyperimmune suppressed B cells	3	M	0.57, 0.32, 1.3	400, 370, >5,000(30)
			L	(0.056, 0.011, 0.017, 0.20) (0.31, 0.37, 0.34, 0.32, 0.44) (0.49, 0.59, 0.24)	(29, 21, 80, 21) (320, 53, 10, 690, 2600) (490, 730, 650)

* Recipients became pregnant within 7 d after cell transfer.
‡ In the radioimmunoassay for CRI$_A$.
§ Cells from A/J mice hyperimmunized with KLH-Ar.
¶ Cells from idiotypically suppressed, hyperimmunized A/J mice.
‖ Numbers in parentheses indicate the percentage inhibition caused by 5,000 ng of anti-Ar antibody.

Offspring were immunized and bled on the same days but with 25 μg of KLH-Ar in CFA. All inoculations were intraperitoneal. Approximately 40% of the offspring survived; a large part of the attrition was due to rejection of litters by their mothers. Controls were carried out (Table I) in which no cells were transferred (group 1) or in which the cells transferred came from A/J mice that had been hyperimmunized with KLH-Ar and allowed to rest for 8–12 wk, but had not been idiotypically suppressed (groups 2 and 6). The top line for each group shows the data obtained for the female parent or parents of that group.

The mothers and offspring in each of the control groups (1, 2, and 6) all produced anti-Ar antibodies that contained a high content of CRI$_A$, as evidenced by the low concentrations of anti-Ar antibody required to cause 50% inhibition in the radioimmunoassay for CRI$_A$. In contrast, mothers that received 2.5 × 10⁶ (group 3) or 1 × 10⁷ (group 4) enriched T cells from HIS mice were highly suppressed with respect to expression of CRI$_A$, although they all produced substantial titers of anti-Ar antibodies. All of the 14 offspring in these two groups were similarly suppressed with respect to production of CRI$_A$. The dose dependency of the suppression is indicated by the data for group 5; 5 × 10⁵ HIS T cells were not suppressive in mothers or their offspring.

The degree of suppression induced by adoptive transfer of B cells from HIS mice was much less profound. 2.5 × 10⁶ B cells caused partial suppression of CRI$_A$

expression in 2 of the 4 female parents (group 7) and in only 2 or 3 of the 12 offspring. Somewhat greater suppressive effects are observed in group 8, in which 1×10^7 B cells from HIS mice were transferred. All 3 of the mothers were significantly suppressed, but 6 out of 12 offspring were not suppressed with respect to CRI_A expression. A comparison of the offspring in groups 3 and 4 with those of groups 7 and 8 indicates that HIS B cells have much less suppressive activity than HIS T cells.

The data in Table II indicate that the suppression of CRI_A observed in neonatal mice was not induced by factors transmitted in milk. Four normal litters were transferred within 24 h after birth to four surrogate mothers that had been given 2.5 $\times 10^6$ T cells from HIS donor mice 3–4 wk earlier. The surrogate mothers were lactating because they had recently given birth to their own litters. Each of these foster mothers was idiotypically suppressed (Table II). It is evident that normal mice nursed by the idiotypically suppressed females produced anti-Ar antibodies with a high content of CRI_A.

The data in Table III demonstrate that sera from HIS mice contain a factor or factors that can passively transfer a state of suppression with respect to the production of CRI_A. In addition, suppression induced in this way in female parents was transmitted to many of the offspring in their litters. Mothers received serum 5–7 d before parturition. Mothers and offspring were then immunized as described above.

As a control, the two mothers of the first group in Table I received 0.2 ml of serum intravenously from hyperimmunized but nonsuppressed A/J mice. The donors had been rested for 8 wk before the transfer. Neither the mothers nor their offspring were idiotypically suppressed. In contrast, 3 of 4 mothers that received 0.2 ml of serum from HIS mice (group 2) were partially or completely suppressed with respect to CRI_A production and 10 of 15 neonatal mice in the four litters were similarly suppressed.

The possibility was considered that the suppressive agent is an Ig. That the active substance is rabbit anti-Id, used to suppress the donor mice, seemed unlikely because the anti-Id had been administered at least 12 wk before the passive transfer of serum and the $t_\frac{1}{2}$ of rabbit IgG in mice is about 6 d (14). Mouse anti-Id could not be detected

TABLE II

Production of CRI_A in Neonatal Mice Raised by Idiotypically Suppressed Foster Mothers

Group	Day 28	
	Anti-Ar titer	Anti-Ar Ab required for 50% inhibition*
	mg/ml	*ng*
Foster mothers (4)	2.4, 0.7	>5,000(18),‡ >5,000(19)
	1.5, 0.011	>5,000(14), 3,200
Litters	(0.032, 0.50, 0.43)	(<10, <10, 20)
	(0.45, 0.27)	(15, 10)
	(0.030, 0.26, 0.81, 0.15)	(<10, <10, 20, <10)
	(0.22, 0.13, 0.25)	(<10, 50, <10)

* In the radioimmunoassay for CRI_A.
‡ Numbers in parentheses indicate the percentage inhibition caused by 5,000 ng of anti-Ar antibody.

TABLE III

Suppressive Effects in Female Parents and Their Litters of Serum from Idiotypically Suppressed Mice

Group	Serum transferred	Volume of serum*	Treatment of serum	Number of litters	Mothers (M) or litters (L)	Day 28	
						Anti-Ar titer	Anti-Ar Ab required for 50% inhibition‡
		ml				*mg/ml*	*ng*
1	Hyperimmune	0.2	none	2	M	0.021, 0.70	61, 58
					L	(0.024, 0.071)	(10, 10)
						(0.31, 0.022)	(21, 43)
2	Hyperimmune suppressed	0.2	none	4	M	0.084, 0.92, 0.28, 1.2	250, 360, 10, >5,000(36)§
					L	(0.051, 0.034)	(29, 175)
						(0.18, 0.20, 0.22, 0.24, 0.21)	(200, >5000[43], >5000[46], 22, 750)
						(0.10, 0.13, 0.26, 0.09)	(10, 380, >1300[0], 2900)
						(0.027, 0.055, 0.12, 1.1)	(1000, >1000[17], 52, 50)
3	Hyperimmune suppressed	0.2	Adsorbed with anti-mouse Fab	2	M	0.020, D¶	>5000(8), D
					L	(0.57, 0.39, 0.26, 0.40)	(>5000[14], >5000[48], 2500, >5000[14])
						(0.78, 0.13, 0.18)	(440, >5000[4], >5000[9])
4	Hyperimmune suppressed	0.2	Adsorbed with anti-rabbit IgG	2	M	0.011, 0.062	>5000(8), >5000(9)
					L	(0.021, 1.5)	(>5000[0], >5000[5])
						(0.14, 0.27, 0.63, 0.26, 0.73)	(>5000[14], >5000[4], >5000[12], 3300, >5000[17])
5	Hyperimmune suppressed	0.02	none	2	M	0.44, 0.75	1400, 31
					L	(0.2, 0.14)	(1000, 42)
						(0.06, 0.033, 0.035)	(31, 36, 22)

* The serum was transferred to pregnant mice from HIS or hyperimmune A/J mice 5–7 d before parturition.
‡ In the radioimmunoassay for CRI$_A$.
§ Numbers in parentheses show the percentage inhibition by the amount of antibody specified.
¶ Deceased.

in the HIS sera by a radioimmunoassay that could detect 50 ng/ml of monoclonal mouse anti-CRI$_A$.

Further evidence that the suppressive agent is not Ig was obtained by adsorption experiments. These experiments were carried out by using Sepharose columns to which specifically purified rabbit anti-mouse Fab or an IgG fraction of goat anti-rabbit IgG was conjugated. The latter reagent was found to have activity against both rabbit Fab and Fc. In each adsorption, ^{125}I-labeled Ig was added to ensure that the column had sufficient capacity to remove all of the Ig. In the adsorptions with anti-mouse Fab, the radiolabeled material was an IgG1κ monoclonal antibody, R16.7. 96% of the radiolabel was retained on the column during the adsorption. Control experiments, carried out separately, showed that the 4% that was not retained was not associated with Ig molecules; <0.4% of the radioactivity initially present was removed by passage through another similar column.

yThe goat anti-rabbit IgG removed 88% of ^{125}I-labeled rabbit IgG that was added to the serum before passage through the column. In control experiments, passage through a second similar column removed only 0.5% of the radioactivity initially present.

It is evident from Table III, groups 3 and 4, that the adsorbed HIS sera retained their capacity to cause suppression of CRI$_A$ in female parents and their offspring. The degree of suppression caused by the adsorbed sera actually seems somewhat stronger than that observed with unadsorbed HIS serum (group 2).

The extent of removal of radiolabeled markers, added during the adsorptions described above, indicated that >95% of the relevant Ig (mouse or rabbit IgG) had

TABLE IV

Suppression of CRI$_A$ in Neonatal Mice Given Serum from Idiotypically Suppressed Hyperimmune Mice

Serum administered*	Number of litters	Day 28	
		Anti-Ar titer	Anti-Ar Ab required for 50% inhibition‡
		mg/ml	*ng*
Hyperimmune	1	0.22, 0.25, 0.14	10, <10, 50
Hyperimmune suppressed	2	(0.21, 0.11, 0.09) (0.14, 0.07, 0.06, 0.24)	(1700, 230, 280) (2200, 900, 340, 1800)

* A/J mice were given 0.2 ml of serum on the day of birth, immunized with 25 µg KLH-Ar intraperitoneally on days 5 and 19, and bled on day 28.

‡ In the radioimmunoassay for CRI$_A$.

been removed. Nevertheless, the possibility was considered that an Ig is actually the suppressive factor but that very small amounts, which escaped adsorption, might be effective. The results obtained with group 5 indicate that this is not the case. When 0.02 ml, rather 0.2 ml, of HIS serum was transferred, with 0.2 ml of normal A/J serum as carrier, one of two female parents was suppressed, but four of five of the offspring in the two litters were not suppressed. These results indicate that even if 10% of the Ig (mouse or rabbit) had escaped adsorption, it would not have been sufficient to cause the suppression observed in groups 3 and 4.

The data in Table IV indicate that HIS serum was suppressive when inoculated into neonatal mice, which were then hyperimmunized. The serum was injected within 24 h after birth. The mice were inoculated with 25 µg KLH-Ar on days 5 and 19 and were bled on day 28. All seven of the mice were suppressed with respect to CRI$_A$ production. Assuming an average value of 30 ng for the amount of anti-Ar antibody required for 50% inhibition for control mice, the degree of suppression was >80% in all of the seven neonatal mice, and was ≥97% in four of the mice.

Discussion

Previous studies (4, 5) have shown that A/J mice that are suppressed by inoculation of rabbit anti-CRI$_A$, hyperimmunized, and permitted to rest for 8–12 wk (HIS mice), possess T cells with anti-Id receptors that can selectively suppress CRI$_A$ formation when adoptively transferred into naive syngeneic recipients. In the present investigation, the cells and sera of HIS mice were tested for their capacity to affect CRI$_A$ formation in neonatal mice when the cells were given to their mothers before mating or when HIS serum was passively transferred to mothers before parturition. In addition, the direct suppressive effects of HIS serum upon recipients were studied.

The results indicate, first, that enriched T cells from HIS mice caused the suppression of CRI$_A$ production not only in the female recipients but also in virtually all of their offspring. 2.5 × 10^6 or 1 × 10^7 cells were effective; 5 × 10^5 HIS T cells were insufficient to cause this effect. Although some suppression was observed with enriched HIS B Cells, they were considerably less potent than the T cells. From the present data, we cannot distinguish between the possibilities that the B cells were suppressive or that the observed effect was due to contaminating T cells.

Experiments in which nonsuppressed neonatal mice were nursed by suppressed, lactating foster mothers indicated that the observed suppression was not attributable to factors transmitted in milk. That milk can contribute to a state of idiotypic suppression was shown by Weiler et al. (15), who observed that a larger percentage of the offspring of idiotypically suppressed female parents were suppressed if they were nursed by idiotypically suppressed females.

It was found that the sera of HIS mice contain a potent suppressor factor that can induce suppression of CRI_A in adult or neonatal mice upon adoptive transfer. Also, when HIS serum was administered to female mice 5–7 d before parturition, most of their offspring were idiotypically suppressed. All of the female parents and their offspring were able to synthesize anti-Ar antibodies when immunized with KLH-Ar; in most of the mice these antibodies did not express CRI_A. Adsorption with immobilized anti-Ig reagents provided evidence that the suppressive activity was not due to mouse or rabbit Ig present in the serum that was transferred.

Maternally transmitted suppression of allotypes (16–18) and idiotypes (15, 19–22) has been reported, but has not previously been attributed to T cells or nonimmuno-globulin factors. We have already mentioned a study that showed an effect of milk from idiotypically suppressed female parents. The present results indicate that suppression mediated by T cells can be transmitted from mother to offspring by a mechanism that does not require milk.

The mechanism by which T cells, transferred into the female parent, caused suppression in their offspring is not apparent. The absence of graft-vs.-host reactions in F_1 mice indicates that few T cells are transferred through the placenta, although there is evidence that some transfer of cells (cell types unidentified) can take place (23). The latter report is, however, controversial (22). Since, as shown by the present data, the sera of HIS mice contain idiotype-suppressor factors, and HIS T cells can elaborate such factors in tissue culture (6), it seems possible that the transferred T cells secreted suppressor factors that were then transmitted to offspring.

It is similarly uncertain how the suppressive effect is transmitted to offspring after passive transfer of HIS serum to the female parent. Perhaps the simplest hypothesis is that the suppressor factor(s) are able to cross the placenta. However, it has frequently been demonstrated that suppressor factors can stimulate suppressor T cells (24–26). A direct answer to the question of whether the suppressor factor can cross the placenta may require the availability of purified, radiolabeled material.

It should be of interest to ascertain whether suppressor factors with other types of specificity (e.g., antigen specific) can act across the placental barrier. The possible presence of idiotypic or antiidiotypic receptors on the factor in HIS serum, as well as its biochemical properties, is being investigated.

Summary

Mice that are suppressed with respect to an idiotype (CRI_A) present in A/J anti-p-azophenylarsonate antibodies, hyperimmunized, and allowed to rest were previously found to possess high concentrations of suppressor T cells with anti-idiotypic receptors. We have now observed that the sera of such mice contain soluble factors that can selectively suppress the CRI_A component of a humoral response when passively transferred to adult or neonatal recipients. When T cells from suppressed, hyperimmunized mice were transferred into female mice before mating, their offspring, upon

immunization, produced anti-Ar antibodies that lacked CRI_A. A state of idiotypic suppression was also produced in offspring when the mother was inoculated with serum from suppressed mice a few days before parturition. The results indicate that the suppressor factor is not an immunoglobulin.

References

1. Hart, D. A., A. L. Wang, L. L. Pawlak, and A. Nisonoff. 1972. Suppression of idiotypic specificities in adult mice by administration of antiidiotypic antibody. *J. Exp. Med.* **135:**1293.

2. Pawlak, L. L., D. A. Hart, and A. Nisonoff. 1973. Requirements for prolonged suppression of an idiotypic specificity in adult mice. *J. Exp. Med.* **137:**1442.

3. Nelles, M. J., L. A. Gill-Pazaris, and A. Nisonoff. 1981. Monoclonal anti-idiotypic antibodies reactive with a highly conserved determinant on A/J serum anti-*p*-azophenylarsonate antibodies. *J. Exp. Med.* **154:**1752.

4. Owen, F. L., S-T. Ju, and A. Nisonoff. 1977. Binding to idiotypic determinants of large proportions of thymus derived lymphocytes in idiotypically suppressed mice. *Proc. Natl. Acad. Sci. U. S. A.* **74:**2084.

5. Owen, F. L., S-T. Ju, and A. Nisonoff. 1977. Presence on idiotype-specific suppressor T cells of receptors that interact with molecules bearing the idiotype. *J. Exp. Med.* **145:**1559.

6. Hirai, Y., and A. Nisonoff. 1980. Selective suppression of the major idiotype component of an antihapten response by soluble T cell-derived factor with idiotypic or antiidiotypic receptors. *J. Exp. Med.* **151:**1213.

7. Tung, A. S. S-T. Ju, S. Sato, and A. Nisonoff. 1976. Production of large amounts of antibodies in individual mice. *J. Immunol.* **116:**676.

8. Dohi, Y., and A. Nisonoff. 1979. Suppression of idiotype and generation of suppressor T cells with idiotype-conjugated thymocytes. *J. Exp. Med.* **150:**909.

9. Klinman, N. R., A. R. Pickard, N. H. Sigal, P. J. Gearhart, E. S. Metcalf, and S. K. Pierce. 1976. Assessing B cell diversification by antigen receptor and precursor cell analysis. *Ann. Immunol. (Paris).* **127:**489.

10. Kuettner, M. G., A. L. Wang, and A. Nisonoff. 1972. Quantitative investigations of idiotypic antibodies. I. Idiotypic specificity as a potential genetic marker for the variable regions of mouse immunoglobulin polypeptide chains. *J. Exp. Med.* **135:**579.

11. Roos, D., and J. A. Loos. 1970. Changes in the carbohydrate metabolism of mitogenically stimulated human peripheral lymphocytes. I. Stimulation by phytohaemagglutinin. *Biochim. Biophys. Acta.* **222:**565.

12. Mage, M. G., L. L. McHugh, and T. L. Rothstein. 1977. Mouse lymphocytes with and without surface immunoglobulin: preparative scale separation in polystyrene tissue culture dishes coated with specifically purified anti-immunoglobulin. *J. Immunol. Methods.* **15:**47.

13. Cuatrecasas, P. 1976. Protein purification by affinity chromatography. *J. Biol. Chem.* **245:**3059.

14. Spiegelberg, H. C., and W. O. Weigle. 1965. The catabolism of homologous and heterologous 7S gamma globulin fragments. *J. Exp. Med.* **121:**323.

15. Weiler, I. J., E. Weiler, R. Sprenger, and H. Cosenza. 1977. Idiotype suppression by maternal influence. *Eur. J. Immunol.* **7:**591.

16. Dray, S. 1962. Effect of maternal isoantibodies on the quantitative expression of two allelic genes controlling γ-globulin allotypic specificities. *Nature (Lond.).* **195:**677.

17. Mage, R. 1975. Allotype suppression in rabbits: effects of anti-allotype antisera upon expression of immunoglobulin genes. *Transplant. Rev.* **27:**84.

18. Jacobson, E. B., and L. A. Herzenberg. 1972. Active suppression of immunoglobulin allotype synthesis. I. Chronic suppression after perinatal exposure to maternal antibody to paternal allotype in (SJL × BALB/c)F$_1$ mice. *J. Exp. Med.* **135**:1151.

19. Cosenza, H., M. H. Julius, and A. Augustin. 1977. Idiotypes as variable region markers. Analogies between receptors on phosphorylcholine-specific T and B lymphocytes. *Immunol. Rev.* **34**:3.

20. Bona, C., K. E. Stein, R. Lieberman, and W. E. Paul. 1979. Direct and indirect suppression induced by antiidiotype antibody in the insulin-bacterial levan antigenic system. *Mol. Immunol.* **16**:1093.

21. Bernabe, R. R., A. Coutinho, P.-A. Cazenave, and L. Forni. 1981. Suppression of a "recurrent" idiotype results in profound alterations of the whole B-cell compartment. *Proc. Natl. Acad. Sci. U. S. A.* **78**:6416.

22. Weiler, I. J. 1981. Neonatal and maternally induced idiotypic suppression. *In* Lymphocytic Regulation by Antibodies. C. Bona and P.-A. Cazenave, editors. John Wiley & Sons, New York. 245–267.

23. Tuffrey, M., N. P. Bishun, and R. D. Barnes. 1969. Porosity of the mouse placenta to maternal cells. *Nature (Lond.).* **221**:1029.

24. Waltenbaugh, C., J. Theze, J. A. Kapp, and B. Benacerraf. 1977. Immunosuppressive factor(s) specific for L-glutamic acid^{50}-L-tyrosine^{50}(GT). II. Presence of I-J determinants on the GT-suppressive factor. *J. Exp. Med.* **146**:287.

25. Tada, T., M. Taniguchi, and K. Okumura. 1977. Regulation of the antibody response by antigen-specific T cell factors bearing I-region determinants. *Prog. Immunol.* **3**:369.

26. Sy, M-S., M. H. Dietz, R. N. Germain, B. Benacerraf, and M. I. Greene. 1980. Antigen and receptor driven regulatory mechanisms. IV. Idiotype-bearing I-J$^+$ suppressor T cell factors induce second order suppressor cells which express anti-idiotype receptors. *J. Exp. Med.* **151**:1183.