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Suppression of a "recurrent" idiotype results in profound alterations of the whole B-cell compartment

(maternal effect/B-cell ontogeny/idiotypic network)

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ABSTRACT **The progeny of BALB/c female mice actively immunized with the trinitrophenyl-binding myeloma protein MOPC460 and producing anti-idiotypic antibodies during pregnancy were compared with mice born of normal mothers for several characteristics of B lymphocytes and their precursors. In all cases, maternal anti-idiotypic immunity resulted in the suppression of the expression of that idiotype by immunocompetent cells in the progeny, as shown by limiting-dilution analysis in single clones of mitogen-reactive IgM-secreting cells. At critical concentrations of circulating maternal antibodies, suppression of the antibody idiotype was found to be accompanied by a large increase in the total number of mature small B lymphocytes. This increase can be accounted for by the selective expansion of B cells bearing nonimmunoglobulin surface structures crossreactive with a MOPC460 idiotope recognized by a monoclonal antibody. In addition, the large majority of newly formed mature B lymphocytes, as well as a large fraction of immunoglobulin-negative cells in the bone marrow of suppressed mice, bear such nonimmunoglobulin MOPC460 crossreactive determinant(s). These results suggest that the suppression of a given "recurrent" idiotype has profound consequences for a large part of the immune system.**

Some antibody idiotypes in the mouse being "recurrent," the possibility exists of studying their suppression in normal animals. This has been achieved by treatment with anti-idiotypic antibodies (1, 2) and by the transfer of cells from animals previously suppressed with antibodies (3). The mechanisms involved in idiotypic suppression are thought to reflect physiological regulatory processes and may be useful in the control of undesirable reactivities. Suppression of idiotype expression has also been observed in the progeny of females immunized with that idiotype (4).

These observations have invariably been interpreted as specific suppression of one or a few idiotype-positive clones, assuming a simplistic mechanism of suppression, whereby idiotype-bearing antibody receptors on competent B cells or their precursors are the sole targets for the "suppressive" anti-idiotype antibodies or cells. Network views of the immune system (5), on the other hand, suggest that suppression of some clones necessarily results in larger perturbations in the steady state of the system. Furthermore, we have recently found that some anti-idiotypic antibodies to "recurrent" idiotypes recognize and trigger into proliferation B cells and their precursors via interactions with polyclonally distributed nonimmunoglobulin membrane structures that function as growth receptors (6).

We have studied the suppression of a combining-site-related idiotope of the trinitrophenyl (TNP)-binding myeloma protein MOPC460 identified by a monoclonal antibody (7). Such an idiotype is recurrent in BALB/c mice, as it can be detected in a variable proportion of antibodies produced by every mouse of this strain (8) and is also expressed on nonimmunoglobulin membrane structures on cells of the B-cell lineage (6).

We report here the possibility of inducing neonatal suppression of the MOPC460 idiotype in a completely syngeneic system by the anti-idiotypic immunity of mothers during pregnancy and the finding that such an idiotypic suppression is accompanied by profound alterations in the development of a large part of the B-cell system.

MATERIALS AND METHODS

Mice. BALB/c mice 1 to >20 weeks old were obtained from the Pasteur Institute (Paris) or from the Institute for Biomedical Research (Füllinsdorf, Switzerland). Normal or immunized BALB/c females were mated with normal BALB/c males and the progeny were studied over an 8-week period.

Anti-Idiotypic Antibodies. A monoclonal anti-BALB/c idiotopic antibody, clone F6(51), described by Buttin *et al.* (7), was used for determination of the idiotype.

Immunization and Testing. MOPC460 females were immunized with the MOPC460 myeloma protein (9) and bled at various intervals. The presence of anti-MOPC460 activity was quantitated by a direct hemagglutination test using MOPC460-coupled sheep erythrocytes (SRBC) as indicator cells (10). Routinely, sera were previously absorbed on SRBC for 30 min at 4°C.

Limiting-Dilution Analysis. Frequency analysis of anti-TNP- or MOPC460 idiotype-producing clones was performed with the lipopolysaccharide (LPS)-reactive population as described by Andersson *et al.* (11). Spleen cell suspensions were cultured in RPMI 1640/2 mM glutamine/10 mM HEPES/50 μM 2-mercaptoethanol/20% fetal calf serum (GIBCO) supplemented with antibiotics and stimulated with LPS (50 μg/ml; *Escherichia coli 055:B5*, Difco), in the presence of rat thymus filler cells at 3×10^6/ml. At each cell dose, 48 replicate cultures were set up and assayed at day 5 for IgM-secreting plaque-forming cells, by using a staphylococcal protein A plaque assay (12), or for cells secreting anti-TNP antibodies, by using TNP-coupled SRBC as indicator cells in the plaque assay (13). Alternatively, cultures were continued to day 10–12 and the supernatants were assayed for the presence of idiotype.

Determination of Idiotype. SRBC were coupled by the chromium chloride method with F6(51) anti-MOPC460 antibody and mixed in V-shaped microtiter plates with culture supernatant at a final concentration of 0.5%. After 2 hr of incubation at room temperature, the number of positive (agglutinated) wells was recorded. Tests were done by using the purified myeloma protein MOPC460 to evaluate the sensitivity of the

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Abbreviations: LPS, lipopolysaccharide; TNP, trinitrophenyl; SRBC, sheep erythrocytes.

technique; positive results were obtained at idiotype concentrations of 10–0.0005 μg/ml. No agglutination was observed when irrelevant myeloma proteins of the same class or type (TEPC15 and MOPC315) were used.

Immunofluorescence. Detection of membrane IgM was done as described (14) using a tetramethylrhodamine isothiocyanate-labeled rabbit anti-mouse μ antibody. Idiotype-like membrane structures were detected by an indirect immunofluorescence technique using TNP-derivatized anti-idiotypic antibodies and a fluorescein isothiocyanate-labeled anti-TNP antibody (15). An anti-[anti-(4-hydroxy-3-nitrophenyl)acetyl] monoclonal antibody (As79), kindly provided by M. Reth (16) was used as a control.

RESULTS

Female BALB/c mice were immunized with MOPC460 protein, mated with normal male mice, and boosted when pregnancy was detected. The offspring from immunized mothers were studied in parallel with age-matched controls, in limiting-dilution analysis for the secretion of MOPC460 idiotype by LPS-reactive IgM-secreting clones. Analysis of mitogen-reactive B cells provides a quantitative description of the immune system with regard to the specific clones detected within that B-cell subset. As the frequency of a given specificity (antibody or idiotype) is determined in the absence of antigen, this analysis is not dependent on a given protocol of immunization but rather reflects the absolute frequency of competent B cells in a given steady state. Individual culture supernatants were assayed in hemagglutination with erythrocytes coated with purified F6(51) anti-MOPC460 monoclonal antibody. As shown in Fig. 1, although normal BALB/c spleens contain a measurable frequency of LPS-reactive B-cell clones producing immunoglobulins recognized by F6(51), mice born from an immunized mother do not. These results demonstrate that idiotypic suppression can be obtained through natural influences in a completely syngeneic situation. Its reproducibility is shown in Table 1, in which a number of independent determinations in mice from different mothers are presented. The suppression is not transmitted to second-generation progeny, as shown by studying the offspring of naturally suppressed females (data not shown).

We have previously described the polyclonal expression on B cells and their precursors of membrane structures that cross-react with recurrent immunoglobulin idiotypes. Conventional anti-MOPC460 idiotype antisera and the F6(51) monoclonal antibody show polyclonal reactivity (6). As the interaction of anti-idiotype antibodies with these receptors leads to proliferation of the target cells *in vitro*, we considered it relevant to evaluate the influences that the maternal anti-idiotype immunity could have at the polyclonal level *in vivo*.

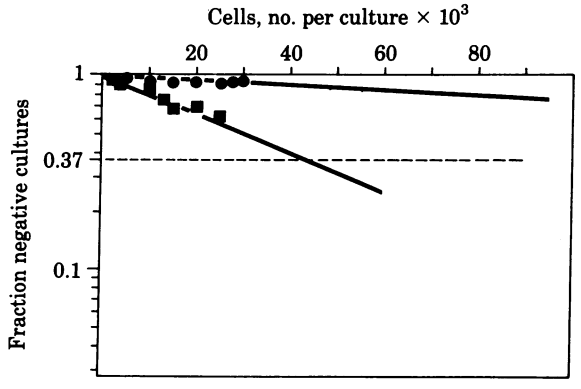

FIG. 1. Spleen cells from normal (■) and neonatally suppressed (●) BALB/c mice were cultured with LPS. Results represent groups of 48 replicates assayed for the presence of idiotype (MOPC460) in the supernatant after 10 days.

Spleen and bone marrow cells from offspring of immunized mothers were studied by immunofluorescence for total number of IgM-bearing mature B lymphocytes and for numbers of cells reacting with F6(51). Another monoclonal antibody against an idiotope on a recurrent anti-(4-hydroxy-3-nitrophenyl)acetyl idiotype that also shows polyclonal reactivity was used as a control to ascertain the specificity of the polyclonal response. As shown in Table 2, spleens of maternally suppressed mice show a marked increase in total number of B cells, as compared with normal age-matched controls. The relative increase is higher at 2 weeks of age (4 times the control value), but it is still present at 6 weeks, when, however, suppressed mice tend to approach the normal counterpart. Spleens of suppressed mice also contain a much higher number of B cells stained by F6(51). Roughly one-third to one-fourth of B cells in spleens of normal 2- to 6-week-old mice are positive for this marker, whereas >80% are positive in 2-week-old suppressed mice. In the total spleen cell population, this represents a 10-fold increase in number of cells. Most important, the increase in total number of B cells is exclusively due to the selective expansion of the subset of cells that express membrane-bound MOPC460-like determinants in addition to IgM. The absolute number of B cells that do not express this marker, constituting roughly 2/3 of the total B cells in normal mice, is unaffected in the suppressed individuals. These alterations become less marked with age, and by 6 weeks, normal and suppressed mice are comparable.

The same pattern is observed in bone marrow, in which mature B cells are 2 to 3 times more frequent in suppressed than in control mice (Table 3). Among such IgM-positive cells, the ones expressing MOPC460-like structures are abundantly represented in suppressed mice and, in 2-week-old animals, account for all B cells. Different from the spleen, however, bone marrow is still abnormal at 6 weeks of age; this can be explained either by a preferential concentration of such "MOPC460-like" B cells in bone marrow or by a shorter lifespan for this B-cell subset. Bone marrow also contains a large population of immunoglobulin-negative cells that are recognized by reagents to recurrent idiotypes; this cell population includes B-cell precursors that acquire surface IgM and mitogen reactivity while maintaining the expression of the idiotype-crossreactive structures (6). Interestingly, however, there is no difference between suppressed and normal mice within the population of IgM-negative MOPC460 idiotype-positive precursors. All the alterations observed appear to occur, as in the spleen, among the cells that simultaneously express membrane IgM and the relevant idiotype-crossreactive structures. In bone marrow, however, a decrease in precursors positive for an unrelated recurrent idiotope (As79) is observed, for which we have at present no explanation.

The findings obtained by immunofluorescence studies, while suggestive of a selective expansion of a given B-cell subset, demanded confirmation in functional studies. We have previously shown that LPS-reactive B cells and cells expressing nonimmunoglobulin MOPC460-like membrane structures are largely different, with <10% overlap (6). This has been confirmed recently by sequential stimulation with LPS and F6(51) (unpublished results). To investigate the polyclonal influence of maternal anti-idiotype immunity on the LPS-reactive B-cell subset of suppressed mice, we have performed limiting-dilution analysis for total LPS-reactive clones and for the frequency of clones producing anti-TNP antibodies. As shown in Table 4, suppressed and normal mice contain the same numbers of LPS-reactive B cells in spleen. As a much higher number of B cells

Table 1. Frequencies of MOPC460 idiotype-secreting clones among LPS-reactive spleen cells in progeny of normal and MOPC460-immunized BALB/c female mice

Spleen cell donor	Age, weeks	IgM-secreting clones in total spleen cells, no.	MOPC460 idiotype-secreting clones, no.	
			In total spleen cells	In IgM-secreting clones
Experiment 1				
Normal	2	1/40	1/60,000	1/1500
Suppressed	2	1/39	<1/300,000	<1/8000
Experiment 2				
Normal	2	1/35	1/35,000	1/1000
Suppressed	2	1/29	<1/100,000	<1/3400
Immunized mother	>12	1/30	<1/150,000	<1/5000
Experiment 3				
Suppressed	6	1/67	<1/250,000	<1/3500

is present in spleens of suppressed mice, this results in a frequency of LPS-reactive cells that is one-fourth to one-third that in the total B-cell population. It can be concluded, therefore, that the expansion of the B-cell compartment in these individuals is not random but selective for LPS-unreactive B cells expressing polyclonally MOPC460 idiotype. It is not surprising, therefore, to find that the frequencies of anti-TNP-secreting clones in the LPS-reactive subset is also normal. It should be pointed out, however, that the suppression of the MOPC460 idiotype on antibodies is general and also involves the LPS-reactive cell population (see also Table 1).

Whatever the mechanism for the polyclonal perturbation described here is, it is related to anti-idiotype immunity and possibly dependent on the level of anti-idiotype antibodies in the mother during pregnancy. We have, therefore, compared the progeny of females having different titers of circulating antibodies during the last 2 weeks of pregnancy. As shown in Table 5, the polyclonal influences were markedly different, depending on the titers of maternal anti-idiotype antibodies. Although low and intermediate titers result in a large increase in the total number of B cells in spleen, which is selective for the subset expressing MOPC460 idiotype, high titers fail to increase the numbers of B cells and actually suppress the MOPC460 subset. The same pattern applies to bone marrow cells (IgM and MOPC460 idiotype-positive). In addition, distinct consequences are recorded among immunoglobulin-negative precursors expressing the MOPC460 idiotype; thus, low titers do not influence this population at all, intermediate titers expand it, and very high titers suppress it. It has to be stressed, however, that regardless of the anti-idiotype titer in the maternal serum, in all cases, the animals were suppressed for the expression of the MOPC460 idiotype on immunoglobulin molecules.

Table 2. MOPC460-crossreactive membrane structure on spleen cells of normal and MOPC460-suppressed mice

Age, weeks	Spleen cell donor	% total cells			% B cells positive	
		anti μ^+	anti μ^+ F6 (51)$^+$	anti μ^+ F6 (51)$^-$	With F6 (51)	With As79
2	Normal	10.4	3.1	7.3	29.8	17.3
	Suppressed	39.8	33.3	6.5	83.6	25.1
3	Normal	30.4	7.3	23.1	24.1	40.7
	Suppressed	44.3	22.2	22.1	50.1	9.0
4 to 5	Normal	28.3	7.3	21.2	25.8	ND
	Suppressed	57.2	27.8	29.7	48.0	ND
5 to 6	Normal	35.6	10.0	25.6	28.1	31.7
	Suppressed	44.3	15.3	28.7	35.3	46.9

ND, not done.

DISCUSSION

The present experiments demonstrate the profound influence of anti-idiotypic immunity on the antibody and B-cell repertoires of the developing immune system. Here, we have unbalanced the steady-state conditions of BALB/c mice to exaggerate the reactivity against a single idiotype and to expand the corresponding anti-idiotypic components. As, however, a network of idiotypes and combining sites cannot be avoided in the normal system, we conclude that such interactions can provide the basis for the selection of available repertoires in all situations.

Our results confirm the possibility of modulating immune repertoires via maternal influence (17–20) and, in particular, of suppressing recurrent idiotypes in newborns by specifically immunizing the mother with that idiotype (4). In our case, suppression was achieved in completely syngeneic conditions and was demonstrated in the absence of any further manipulation of the progeny, such as specific immunization. This excludes from this regulation any antigenic determinant other than idiotype, as well as any possible role of antigenic exposure in the establishment of the suppressed state. These characteristics of the experimental system would appear, therefore, to be closer to physiological conditions.

Detectable immunity against self-idiotypic determinants on a single myeloma protein results in developmental perturbations encompassing a large part of the whole B-cell compartment. These findings go beyond the information available to date on idiotypic suppression and support network concepts, in which exacerbation of the anti-idiotypic activity of some clonal specificities is expected to result in the perturbation of many other clones, possibly of the whole antibody repertoire (5). The fact that idiotypic suppression was obtained via maternal influence is probably not determinant for our findings, except for the critical time in ontogeny when the system is exposed to anti-idiotypic immunity (21). Similar findings, therefore, may be expected in other experimental conditions, such as neonatal

Table 3. MOPC460-crossreactive membrane structures on bone marrow cells of normal and MOPC460-suppressed mice

Age, weeks	Bone marrow cell donor	% total cells				
		μ^+	μ^+F6 (51)$^+$	μ^+As79$^+$	μ^-F6 (51)$^+$	μ^-As79$^+$
2	Normal	3.7	1.5	1.8	32.8	47.6
	Suppressed	8.5	8.5	0.67	34.7	6.8
3	Normal	4.7	1.9	2.2	15.3	29.4
	Suppressed	12.8	9.0	1.5	25.3	7.8
5 to 6	Normal	5.9	1.8	3.3	27.3	14.0
	Suppressed	8.1	6.9	4.5	22.7	32.8

Table 4. Absolute frequencies of anti-TNP- and MOPC460 idiotype-secreting clones among LPS-reactive B cells in 2-week-old BALB/c mice born from immunized females and in age-matched controls

Spleen cell donor	Frequency no. Ig-secreting clones/total spleen cells	Anti-TNP clones/ Ig-secreting clones	MOPC460 Id-positive clones/ Ig-secreting clones
Normal	1/40	1/83	1/1500
Suppressed	1/39	1/93	<1/8000
Suppressed	1/20	1/130	ND

Ig, immunoglobulin; Id, idiotype; ND, not done.

suppression by injection of anti-idiotypic antibodies.

Our previous finding that recurrent idiotypes, in this case the one recognized by F6 (51) on MOPC460, crossreact antigenically with nonimmunoglobulin receptors polyclonally distributed in a large set of cells of the B-cell lineage may explain the present results. We have shown that such idiotypic structures are growth receptors and that suitable concentrations of the appropriate anti-idiotypic antibodies induce extensive proliferation of both B cells and their immunoglobulin-negative precursors that will further develop to the expression of surface immunoglobulin and acquisition of immunocompetence (ref. 6; unpublished results). It is likely, therefore, that the present results represent the *in vivo* mitogenic activity of anti-idiotypic antibodies, driving the expansion of a large number of cells along the B-lymphocyte pathway. This hypothesis is supported by the large increase in mature immunoglobulin-positive B cells, which selectively involves the subset of B lymphocytes expressing MOPC460-like growth receptors that, as shown *in vitro*, are the exclusive targets for the mitogenic activity of anti-idiotypic antibodies (6). The argument is reinforced by the dose-dependent effects, which also parallel the *in vitro* situation. Low titers of maternal anti-idiotypic antibodies induce the expansion only of the population of cells expressing both membrane immunoglobulin and MOPC460-like membrane determinants, while higher titers also result in the expansion of immunoglobulin-negative MOPC460-positive precursors and very high titers extensively suppress this subset of B cells, already at the level of immunoglobulin-negative precursors. It would appear that, at low concentration of anti-idiotypic antibodies, expression of surface immunoglobulin is required for stimulation. This could be due either to a lower threshold for activation of immunoglobulin-positive cells or to idiotypic crossreactivity with membrane immunoglobulin. In this case, only precursor cells that, by expressing surface immunoglobulin-sharing idiotopes with growth receptors, will concentrate enough stimulatory anti-idiotypic antibodies, will proliferate.

Table 5. Effect of maternal antibody titers on B-cell system of the progeny

Maternal anti-Id titer	% total cells: Spleen μ^+	Spleen μ^+F6 (51)$^+$	Bone marrow μ^+	Bone marrow μ^+F6 (51)$^+$	Bone marrow μ^-F6 (51)$^+$
0*	14.2	4.2 (29.6)	3.9	1.7 (43.6)	14.5
1/8	46.5	24.6 (52.9)	8.9	7.1 (79.8)	15.0
1/500	43.1	39.3 (91.2)	5.2	4.8 (92.3)	44.0
1/>4,000	36.8	1.3 (3.5)	6.7	0.4 (5.9)	1.7

Values in parentheses represent the percent of idiotype-positive cells out of total μ^+ cells. Id, idiotype.
* Control: age-matched mice from nonimmunized mothers.

This mechanism, which we have considered before (6, 22), will result in increased frequencies of B cells bearing any antibody idiotope that can be expressed on the same immunoglobulin molecule as the target for idiotypic suppression. Moreover, if the postulate that "escape" from anti-idiotypic pressure at the single-cell level is mediated by somatic variation of immunoglobulin idiotopes is correct, we would expect that suppressed animals would contain increased frequencies of mature cells expressing antibody specificities related to MOPC460. This has been found *in vitro* (unpublished results), and it supports a model, based on such idiotypic crossreactivities, for the somatic selection for variants of germline products. We conclude that, in most cases, except at very high levels of anti-idiotypic antibodies, anti-idiotypic immunity is stimulatory rather than suppressive, in terms of the whole B-cell compartment. By definition, however, the target idiotope on immunoglobulin molecules is always suppressed.

In contrast with the subset selectivity of the polyclonal effects of anti-idiotypic immunity, the clonal suppression of the idiotope on antibodies appears to occur in all functionally defined B-lymphocyte subpopulations, as it is observed among LPS-reactive cells, which in turn are not affected by the former alterations. As, however, there are no available methods for panclonal stimulation of B lymphocytes nor are there immunogens that stimulate all clones of a given idiotypic or antibody specificity in the various functional subsets, this conclusion has to be taken as provisional. Recent observations that mice suppressed by neonatal injection of anti-idiotypic antibodies against the major TEPC15 idiotype contain appreciable numbers of idiotype-positive precursor B cells that can respond to Pc-LPS but not to Pc-KLH or to pneumococci (23) reinforce this note of caution.

In conclusion, the present results support the notion that idiotypic suppression operates by positively selecting for variants of the target idiotype, this process taking place by polyclonal expansion of B-cell subsets marked by the expression of idiotype-crossreactive triggering receptors. The profound alterations thus induced must be responsible for the maintenance of the suppressed state after loss of maternal influences, in cases where suppressor T cells cannot be invoked (21).

Although the biological significance of idiotypic growth receptors on B cells and their precursors has not been completely clarified, these results demonstrate their participation in immune networks and their *in vivo* relevance in B-cell development.

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