ISOLATED HAPTEN-BINDING RECEPTORS OF SENSITIZED LYMPHOCYTES

III. Evidence for Idiotypic Restriction of T-Cell Receptors*

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There is now good evidence that variable portions of both T- and B-cell receptors for antigen are encoded by *V* genes in the heavy chain linkage group (1, 2, 3). However, it is not known whether the sets of *V* genes expressed in the two cell compartments are identical or only overlapping. Only a few *V* genes, identified by serological markers of their gene product (idiotypic markers), have been shown to be coexpressed in both T- and B-cell receptors, and indirect evidence from functional and regulatory studies would suggest that in the two cell compartments, the rules by which cells are selected differ according to their receptor specificity (4).

In the present study we approach this question in a more direct way. The humoral antibody response of C57BL/6 mice against the hapten 4-hydroxy-3-nitro-phenylacetyl (NP)[1] is in its initial phase restricted to a family of closely related antibody species which constitute the NP^b idiotype. This idiotype is inherited as a single genetic unit in close linkage to the heavy chain allotype (5, 6). In the secondary immune response, the proportion of antibodies carrying the NP^b idiotype declines from 70–90% to 10–20%, and the antibody population becomes strongly heterogeneous (7, 8). Our technique of receptor isolation from T and B lymphocytes (3, 9–12) allows us to study idiotype expression at the receptor level in parallel. We show here that NP-binding receptors isolated from sensitized B cells are strikingly similar to humoral antibodies in the secondary response in that only a small fraction expresses the NP^b idiotype. In contrast, receptors of putative T-cell origin appear to be restricted to the primary NP^b idiotype in accord with our previous data (3).

Materials and Methods

Animals. C57BL/6 mice and BALB/c mice were obtained from the Zentralinstitut für Versuchstierzucht, Hannover, West Germany, and G1. Bomholtgaard, Ltd., Ry, Denmark. CBA mice were purchased from The Jackson Laboratory, Bar Harbor, Maine, and from S. Ivanovas, Ltd., Kisslegg, West Germany. $(C57BL/6 \times CBA/J)F_2$ hybrids were bred in our animal colony.

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[1] *Abbreviations used in this paper*: cap, ε-aminocaproic acid; CG, chicken gamma globulin; G anti-MIg, goat anti-mouse Ig serum; HPI, haptenated phage inactivation; NIP, 4-hydroxy-5-iodo-3-nitro-phenylacetyl; NP, 4-hydroxy-3-nitro-phenylacetyl; R anti-Id, rabbit antiserum against NP^b idiotype.

Male and female mice, aged 6–8 wk, were vaccinated against ectromelia and rested for at least 2 wk before entry into the experiment.

Antisera and Immunosorbents. The polyspecific goat antiserum to mouse immunoglobulin (G anti-MIg) has been described (3, 9). An anti-idiotypic antiserum against primary anti-NP antibodies (the NP^b idiotype) of C57BL/6 mice was raised in a rabbit. For this purpose, C57BL/6 anti-NP antibodies were induced and purified by affinity chromatography as described previously (5). The rabbit was given an initial intramuscular injection of 100 μg of the antibodies in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) on the shoulder, followed by the injection of 50 μg antibody in incomplete adjuvant 1 mo later. After another 10 days, the rabbit received 50 μg of the antibody preparation i.v., and was bled 20 days after this injection. The resulting antiserum was absorbed on insolubilized normal C57BL/6 serum and MOPC 104e protein as described previously (5). We obtained an anti-idiotypic serum (R anti-Id) which reacted specifically with the majority (>80%) of primary C57BL/6 anti-NP antibodies, but did not bind C57BL/6 normal Ig or antibodies of unrelated specificity. In genetic experiments, the antiserum was shown to detect an idiotypic marker indistinguishable from the one detected by our previous guinea pig anti-idiotypic serum (5) except that the reaction of R anti-Id with the anti-NP antibodies could be specifically inhibited by 4-hydroxy-5-iodo-3-nitrophenylacetyl caproic acid (NIP-cap). The detailed characterization of R anti-Id is the subject of a separate publication.[2] The specificity of the antiserum, however, is apparent from the data reported in this paper (see Results). Immunosorbents were prepared by coupling the immunoglobulin (Ig) fraction of R anti-Id (prepared by precipitation with ammonium sulfate at 50% saturation) or G anti-MIg whole serum to Sepharose 4B-Cl (Pharmacia Fine Chemicals, Uppsala, Sweden) according to March et al. (13).

Immunizations, Receptor Preparations, Absorptions, and Phage Inactivation Assay (HPI). Preparation of NP_{16} chicken gammaglobulin (NP_{16}-CG), immunization procedures, preparation of hapten-coupled nylon discs, preparations of splenic lymphocytes, isolation of hapten-specific receptor material, absorption techniques, and HPI with NIP-cap-T4 bacteriophage (gift of Doctors O. Mäkelä and M. Becker) have been described previously (3, 9).

Results

The aim of the experiments described here was the determination of the frequency of the NP^b idiotype in NP-specific T- and B-cell receptors from mice carrying the $Ig\text{-}l^b$ allotype. For this purpose mice of various strains were primed with NP-CG, and NP-binding receptor material was isolated from the spleen cells on haptenated nylon mesh as described previously (3, 9).

The receptor material consists of two fractions, one reacting with class- and type-specific anti-Ig sera (anti-Ig^+ fraction), and another lacking determinants of constant Ig domains (anti-Ig^- fraction). Cell separation experiments indicate that the anti-Ig^+ fraction represents B-cell receptors, whereas the anti-Ig^- fraction originates from T lymphocytes (3, 9). The frequency of the NP^b idiotype in the two fractions was determined in a two-step procedure. First, the total receptor preparation was absorbed with insolubilized R anti-Id or insolubilized normal rabbit serum, and the hapten binding activity was determined before and after absorption. Assuming a similar avidity distribution in NP^b idiotype positive and negative molecules, the NP^b idiotype frequency in the total receptor material (anti-Ig^+ and anti-Ig^- fraction) can be calculated from these data. Second, the anti-Ig^- fraction was separated from the total receptor preparation by absorption with insolubilized G anti-MIg serum and the frequency of the NP^b idiotype was determined in this fraction as described above. The combined data also allow the calculation of the idiotype frequency in the anti-Ig^+ fraction.

The results of a typical experiment appear in Fig. 1. In this experiment,

[2] T. Imanishi-Kari and K. Rajewsky. Manuscript in preparation.

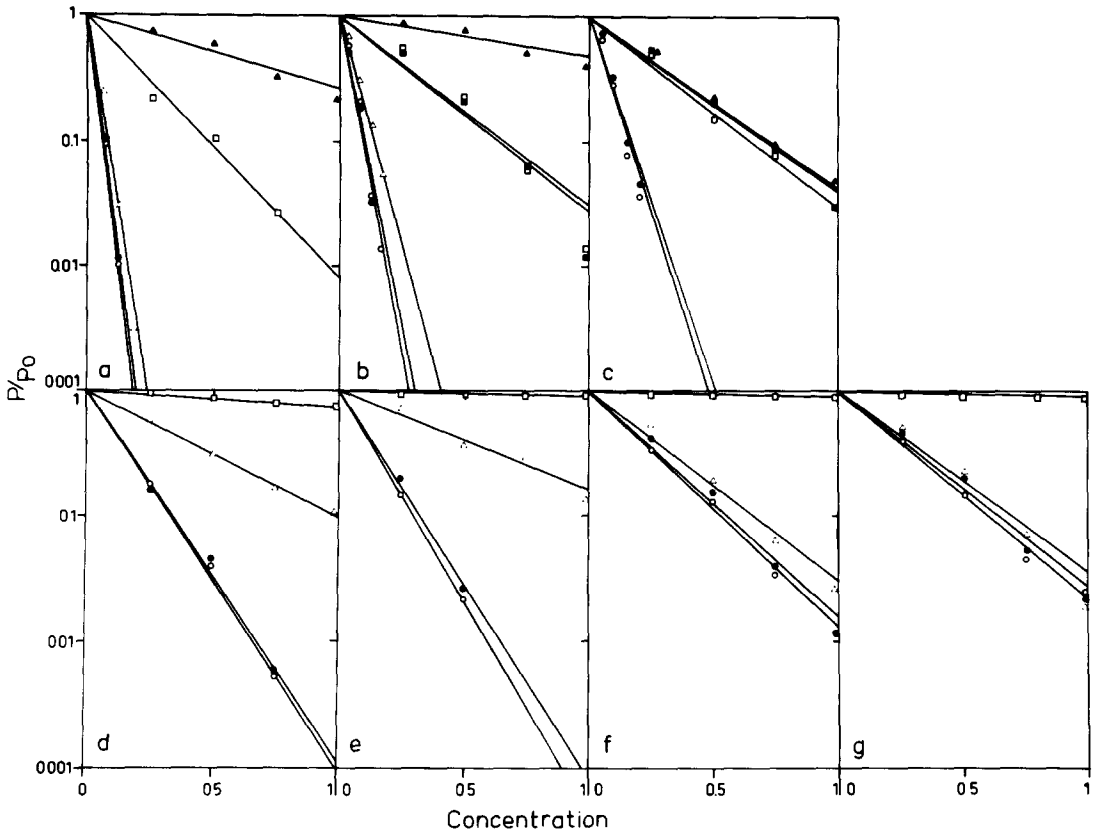

FIG. 1. Analysis of idiotype expression in receptor material and humoral antibodies. The following receptor preparations isolated on NP-cap nylon discs from spleen cells of NP_{16}-CG sensitized mice and anti-NP serum antibodies of the lymphocyte donor strains were analysed: (a) C57BL/6 receptor isolated 6 wk after primary immunization, (b) (C57BL/6 × CBA/J)F_2 receptor isolated 4 wk after primary immunization from animals selected for homozygous Ig-1^b allotype, (c) BALB/c receptor isolated 13 wk after primary immunization, (d) serum pool collected from 10 C57BL/6 mice bled 6 wk after priming, (e) primary C57BL/6 antibodies purified by adsorption to NP-bovine serum albumin-Sepharose and subsequent elution with acidic buffer, (f) serum pool collected from 5 C57BL/6 mice bled 10 days after secondary immunization, (g) serum pool collected from 7 BALB/c mice bled 6 wk after primary immunization. The concentration of phage inactivating material on the abcissa is given in arbitrary units. Inactivation of NIP-cap-T4 bacteriophage (p/p_o, number of bacteriophage plaques in presence of inactivator divided by number of plaques in absence of inactivator) by (○) nonabsorbed material or material absorbed on: (●) Sepharose-bound normal rabbit serum; (□) G anti-MIg immunosorbent; (△) R anti-Id immunosorbent; (■) G anti-MIg immunosorbent in two sequential absorptions steps; (▲) G anti-MIg immunosorbent and subsequently on R anti-Id immunosorbent. Immunosorbents were prepared by coupling whole normal serum, G anti-MIg serum or the IgG fraction of R anti-Id serum to CNBr-activated Sepharose 4B-CL.

receptors from three groups of animals were analyzed. Two receptor preparations originated from mice carrying the Ig-1^b allotype, namely C57BL/6 mice and mice from a (C57BL/6 × CBA)F_2 generation which were homozygous for the Ig-1^b allotype. We know from previous experiments which included a genetic analysis that the anti-Ig^- fraction in these two receptor preparations carries the

TABLE I

Binding of NP-Specific Receptors and Serum Antibodies to G anti-MIg and R anti-Id Immunosorbents

No.	NP-binding material	Total receptor and antibodies		Anti-Ig⁻ fraction‡	Anti-Ig⁺ fraction
		% Absorption by		% Absorption by R anti-Id*	
		G anti MIg	R anti Id		
1	C57BL/6 receptor	85§	20	74	9
2	(C57BL/6 × CBA)F_2 receptor, Ig-$1^{b/b}$	84‖	26	81	13
3	BALB/c receptor	75‖	n.d.¶	4	
4	Primary C57BL/6 anti-NP serum	96§	67		
5	Purified primary C57BL/6 anti-NP antibodies	99§	75		
6	Secondary C57BL/6 anti-NP serum	99§	18		
7	Primary BALB/c anti-NP serum	98§	8		

* Measured directly for anti-Ig⁻ fraction and calculated for anti-Ig⁺ fraction as described in text.

‡ The anti-Ig⁻ fractions in the present study are smaller than in previous experiments. The reason for this discrepancy is not entirely clear but appears to be related to the technique for immunosorbent preparation in that certain types of immunosorbents of anti-Ig sera exhibit substantial nonspecific absorption.

§ Determined by single absorption with G anti MIg and corrected for absorption by normal rabbit serum.

‖ Determined by two sequential adsorptions with G anti-MIg and corrected for absorption on normal rabbit serum.

¶ n.d., not determined.

NP^b idiotype (3). The third receptor preparation was of BALB/c origin and should therefore be negative for the NP^b idiotype (5). Also included in the experiment were primary and secondary serum antibodies from NP-sensitized C57BL/6 mice, and primary anti-NP antibodies of BALB/c origin for control.

From the phage inactivation curves in Fig. 1 we can calculate the fractions of NP-binding activity associated with the NP^b idiotype in the various receptors and antibodies. The results of this calculation appear in Table I.

As expected, the frequency of the NP^b idiotype is high in primary and low in secondary anti-NP sera of C57BL/6 mice. Only 8% of the activity of BALB/c antibodies are absorbed by the anti-idiotypic serum. This must be considered nonspecific since in binding inhibition experiments, the same antibodies are totally negative for the NP^b idiotype (idiotype frequency <1%; T. Imanishi-Kari, unpublished data).

In the receptor preparations, the situation is strikingly different for anti-Ig^+ and anti-Ig^- fractions. In accord with our previous data (3), the bulk of the activity of the anti-Ig^- fraction from Ig-1^b mice is eliminated by the anti-idiotypic immunosorbent. Elimination is specific since it does not extend to anti-Ig^- receptors of BALB/c origin. In contrast, the anti-Ig^+ receptor fraction from Ig-1^b mice appears to contain very few, if any, NP-binding molecules carrying the NP^b idiotype. This result is new and allows us for the first time to

distinguish the anti-Ig$^-$ receptor fraction positively from the anti-Ig$^+$ fraction by a marker which is strongly expressed in the former, but not expressed, or scarcely detectable in the latter fraction. In our previous experiments (3), an anti-idiotypic serum was used which allowed the determination of the NP^b idiotype only in anti-Ig$^-$ receptors.

Discussion

The present study confirms by absorption analysis that the NP^b idiotype is expressed on the majority of primary anti-NP antibodies of C57BL/6 mice, but that it represents only a minor component of the antibody population in the secondary response (7, 8). Receptors on memory B cells in NP-sensitized animals should therefore be largely devoid of the NP^b idiotype. This is experimentally approached in our analysis of the anti-Ig$^+$ receptor fraction. The discovery of very low idiotype frequency in this material strongly supports our interpretation that the anti-Ig$^+$ fraction represents receptors of sensitized B lymphocytes. Since the majority of the anti-NP antibodies circulating in the animals from which the lymphocytes for receptor preparation are recovered carry the NP^b idiotype, the anti-Ig$^+$ receptor fraction cannot be passively adsorbed antibody.

In striking contrast to the anti-Ig$^+$ fraction, the majority of the anti-Ig$^-$ receptors carry the NP^b idiotype as shown in the present and a previous study (3). The anti-Ig$^-$ fraction is considered to represent T-cell receptors on the basis of cell fractionation experiments (3, 9). Previous evidence argues against the possibility that this material is passively adsorbed conventional antibody in that the material does not appear to carry antigenic determinants of any of the known immunoglobulin constant domains (3, 11). Another strong argument comes from recent experiments with SJL mice.[3] These animals carry the Ig-1^b allotype, but do not detectably express the NP^b idiotype at the antibody level, presumably because of their low expression of lambda light chains which are associated with antibody molecules carrying the NP^b idiotype (5, 8, 14). NP-binding receptors isolated from sensitized SJL mice do not detectably express the NP^b idiotype in the anti-Ig$^+$ fraction, but most of the activity in the anti-Ig$^-$ fraction is specifically absorbed by the anti-idiotypic serum.

It would thus appear that in the present system, V_H gene expression in T-cell receptors is largely restricted to the major idiotype of the primary anti-NP antibody response. The extent of this restriction is not clear at present. The T-cell-receptor repertoire might become more heterogeneous still later in the immune response, and we do not know whether the receptor molecules carrying NP^b idiotypic determinants are equally heterogeneous as the corresponding antibody molecules in the primary response (15).

The present experiments raise the question whether as far as V_H-gene expression goes, T cells are restricted to the expression of only a few genes which appear at the antibody level as major idiotypes, and which, there is good reason to believe, are encoded in the germ line. B cells may initially express the same genes in their receptors, but then go through a diversification process in ontog-

[3] U. Krawinkel, M. Cramer, I. Melchers, T. Imanishi-Kari, and K. Rajewsky. Manuscript in preparation.

eny. This would be in line with experimental results suggesting that the affinity of T-cell receptors for antigen does not increase in the course of an immune response, in contrast to the affinity of B-cell receptors (16, 17). It is tempting to ask whether the preferential recognition by T cells of structures encoded by the major histocompatibility complex might correspond to properties of the V^H domains to the expression of which T cells appear to be restricted. Experiments along these lines are in progress.

Summary

The primary antibody response of C57BL/6 mice to the 4-hydroxy-3-nitrophenylacetyl (NP) hapten is restricted to antibody molecules expressing the NP^b idiotype. This idiotype is a genetic marker for V genes in the heavy chain linkage group. In the secondary response, the frequency of NP^b idiotype-positive molecules within the antibody population drops to very low values. Accordingly, isolated NP binding receptors from NP-sensitized B lymphocytes are largely devoid of this idiotype. In contrast, the NP^b idiotype is expressed on the majority of the receptor fractions which we consider T-cell derived. This finding suggests that the antigen receptors of T lymphocytes may be restricted to the expression of major (germ-line encoded?) heavy chain idiotypes.

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