Brief Definitive Report

HELPER T CELLS REACTING TO IDIOTYPE ON IgG BUT NOT IgM

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The administration of antiidiotypic or idiotypic antibody to a mouse can result in idiotype enhancement or suppression, depending on the experimental conditions (reviewed in 1–4). We have previously (5) demonstrated that, in the case of idiotypes associated with the anti-(4-hydroxy-3-nitrophenyl)acetyl (NP) response of C57BL/6 mice, regulation by antiidiotype predominantly affects the IgG (as opposed to the IgM) response and, within this response, a certain, idiotypically defined subset of anti-NP antibodies. At least in the case of chronic suppression, regulatory T cells are involved in mediating the effect (5). Here we demonstrate that immunization with idiotypic antibody of the IgG1 but not the IgM class induces T helper cells that appear to recognize the same subset of IgG1 anti-NP antibodies and enhance their expression in an anti-NP response.

Materials and Methods

Mice. C57BL/6 mice were from our animal facility.

Antibodies. The monoclonal anti-NP antibodies are listed in Table I. The monoclonal antiidiotope antibodies Ac38, Ac146, A39-40, and A6-24 were raised against antibody B1-8 and recognize distinct idiotype determinants (10; note that we use the same designation for an antiidiotope antibody and its target idiotope, i.e., antibody Ac146 recognizes idiotope Ac146, etc.). The purification of these monoclonal antibodies and the preparation of goat anti-mouse IgG1 and IgM antibodies has been described previously (5).

Antigen. Polymerized flagellin (POL) was a gift from Dr. G. Nossal, Melbourne. NP-coupled keyhole limpet hemocyanin (KLH) and (4-hydroxy-5-iodo-3-nitrophenyl)acetyl (NIP)-coupled POL were prepared as described previously (5). Trinitrophenylated KLH (TNP-KLH) was prepared by reacting the protein with 2,4,6-trinitrobenzene sulfonic acid (TNBS) (Sigma, Munich, FRG). Monoclonal anti-NP antibodies were coupled to TNBS in the presence of NP–caproic acid. The hapten was subsequently removed by dialysis. NP binding capacity and idiotope expression of TNP-coupled anti-NP antibodies were almost the same as those of the native molecules, as determined by radioimmunoassay (RIA) (data not shown). Molar substitution ratios were NP_{14}-KLH, NIP_2-POL, TNP_{17}-KLH, $TNP_{59,21}$-B1-8, TNP_{55}-267.7, TNP_6-N1G9, TNP_7-S24-1 and TNP_8-B1-48.

Immunization. For the induction of hapten-specific B cells, C57BL/6 mice were immunized with 100 μg alum-precipitated NP-KLH or TNP-KLH together with *Bordetella pertussis* vaccine (Behringwerke, AG, Marburg/Lahn, FRG). For the induction of idiotype-specific T cells, C57BL/6 mice were primed with 100 μg monoclonal anti-NP antibody (B1-8 or N1G9) emulsified in complete Freund's adjuvant (CFA), subcutaneously at the

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TABLE I

Characteristics of Monoclonal Anti-NP Antibodies Used

Name	Origin	Isotype			NP binding	Idiotype expression				Ref.
		IgH	H	L		Ac38	Ac146	A39-40	A6-24	
B1-8	C57BL/6	b	μ	λ1	+	+	+	+	+	6
267-7	BALB/c	a	μ	λ1	+	−	−	−	−	7
N1G9	C57BL/6	b	γ1	λ1	+	+	+	+	+	8
B1-48	C57BL/6	b	γ1	λ1	+	+	−	−	−	6
S24-1	C57BL/6	b	γ1	λ1	+	−	−	−	−	9

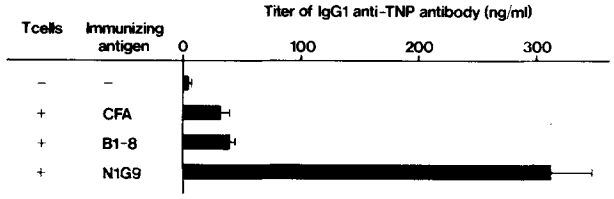

FIGURE 1. Induction of N1G9-specific T helper cells: 3×10^5 Lyt-2$^-$ T cells were cultured with 5×10^5 T cell–depleted, TNP-primed SC in the presence of 100 ng TNP-N1G9/ml in 0.2-ml triplicate cultures. Titers of IgG1 anti-TNP antibody in culture supernatants after 8 d were measured by RIA. Arithmetic mean titers and standard deviations are given.

base of the tail. Mice were boosted with 100 μg idiotype in incomplete Freund's adjuvant (IFA) 4 wk later and, in saline, 8 wk later.

Cell Preparation. T cell–depleted spleen cells were prepared by treatment of spleen cells (SC) twice with monoclonal anti-Thy-1.2 (New England Nuclear, Dreieich/F.R.G.), anti-Lyt-2.2 (gift of Dr. F. W. Fitch, Chicago), and rabbit complement. T cells were purified from the draining lymph nodes of B1-8- or N1G9-primed mice 7 d after the last immunization, by passage over a nylon column followed by panning on goat anti-mouse IgM antibody–coated dishes. The cells were subsequently treated with anti-Lyt-2.2 and complement.

In Vitro Antibody Response and Antibody Assay. Secondary anti-NP response was obtained by stimulation with the type II T-independent (TI-II) antigen NIP-POL. 4×10^6 T cell–depleted SC from NP-KLH-primed C57BL/6 mice were cultured together with 1×10^6 T cells treated with anti-Lyt-2 plus complement, in the presence of 100 ng NIP-POL/ml in 1 ml cultures. For secondary anti-TNP responses, 5×10^5 TNP-primed, T cell–depleted SC were cultured with $1–30 \times 10^4$ T cells in the presence of 100 ng TNP-coupled monoclonal antibody per milliliter in 0.2-ml microtiter wells. 8 d later, titers of anti-TNP, anti-NP, or idiotope-positive antibodies in the culture supernatants were determined by RIA as described previously (5).

Results

Induction of N1G9-specific T Helper Cells. C57BL/6 mice were immunized with two anti-NP antibodies, B1-8 (IgM, λ1) and N1G9 (IgG1, λ1), or with adjuvant alone. The variable (V) regions of the two antibodies are known to be structurally identical and are therefore idiotypically fully crossreactive (8). Lyt-2$^-$ T cells from the immunized animals were analyzed for their ability to provide help for a secondary TNP-specific B cell response in vitro to the antigen TNP-N1G9 (Fig. 1). Good helper activity was obtained only by immunization with N1G9. Even at a fivefold higher immunizing dose of antibody B1-8, there was no significant response (data not shown). When the N1G9-primed T cells were treated with monoclonal anti-Thy-1 or anti-Ly-1 antibodies and complement,

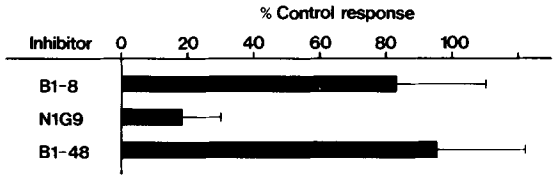

FIGURE 2. Inhibition of the T cell–mediated, in vitro anti-TNP response to TNP-N1G9 by free antibodies: 3×10^5 N1G9-primed, Lyt-2^- T cells were cultured with 5×10^5 T cell–depleted, TNP-primed SC in the presence of 100 ng TNP-N1G9/ml in 0.2-ml triplicate cultures. Free N1G9, B1-48 (20 μg/ml), or B1-8 (100 μg/ml) were added to the culture. 8 d later, titers of IgG1 anti-TNP antibody in culture supernatant were measured. Arithmetic mean titers and standard deviations are given as percent of the mean titer obtained in the absence of inhibitor (255 ± 67 ng/ml).

helper activity was abolished (data not shown). Since the cells do not express surface-bound immunoglobulin (Ig) and the Lyt-2 antigen (see method of preparation), helper activity in this system may be expressed by a single-cell type with the classical T helper phenotype sIg^-, Lyt-2^-, Ly-1^+, and Thy-1^+.

Specificity of N1G9-induced T Helper Cells. The helper T cells recognizing antibody N1G9 (IgG1, λ1) as a carrier could recognize (*a*) an idiotypic determinant as such, (*b*) an idiotypic determinant in association with the heavy chain constant region of IgG1, or (*c*) an IgG1 determinant as such. In the first case one would have to assume that immunization with antibody B1-8 (IgM, λ1; V region identical with that of N1G9) was inefficient for unknown reasons.

In an attempt to assess the specificity of the cells further, we tried to inhibit their activity in the in vitro response to TNP-N1G9 by free antibodies (Fig. 2). Inhibition was obtained with antibody N1G9 but not B1-8 nor B1-48, a λ chain–bearing anti-NP antibody whose isotype is the same as that of N1G9 but which carries a distinct, though related, V region. This result suggests that the helper T cells react to an idiotypic determinant in association with the heavy chain constant region of IgG1. This notion gained further support from experiments in which several monoclonal anti-NP antibodies were coupled to TNP and used as carriers in anti-TNP response. A summary of these experiments is shown in Fig. 3. TNP-specific responses were induced in a dose-dependent fashion in the presence of TNP-N1G9, but not with TNP conjugates of two other λ1 chain–bearing IgG1 anti-NP antibodies, B1-48 and S24-1. This indicates that the helper T cells recognize idiotypic determinants present on N1G9 but not on B1-48 and S24-1. Of the known N1G9 idiotopes only one (Ac38) is shared with B1-48, whereas several others, such as Ac146, A39-40, and A6-24, are absent from both B1-48 and S24-1 (10 and unpublished data) and are thus potential targets of the T helper cells.

However, the results in Fig. 3 again suggest that idiotypic determinants alone are insufficient to trigger optimal T cell help, since antibody B1-8, idiotypically identical to N1G9, but an IgM, is a poor carrier in the system (Fig. 3). This was further substantiated in experiments in which additional TNP conjugates of B1-8 were used (TNP_{21}-B1-8 and TNP_{59}-B1-8) and the antigen dose varied between 1 ng and 1 μg. In all cases TNP-N1G9 induced a much higher response than the TNP-B1-8 conjugates (data not shown). Thus, T cell help appears "restricted"

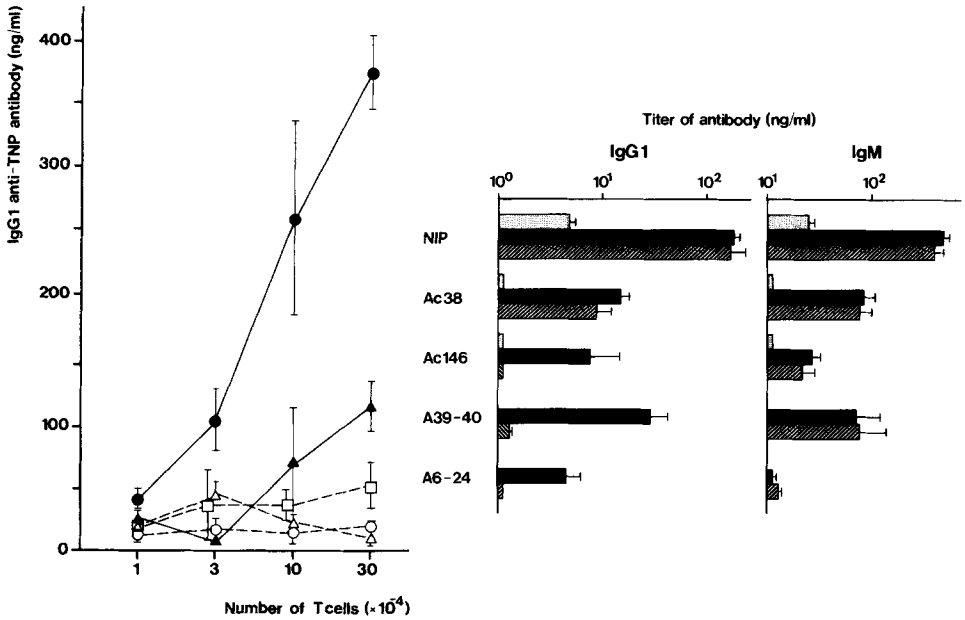

FIGURE 3. Specificity of N1G9-induced T helper cells: Graded numbers of Lyt-2⁻, N1G9-primed lymph node T cells were cultured with 5×10^5 T cell–depleted, TNP-primed SC in the presence of 100 ng antigen/ml in 0.2-ml triplicate cultures. Antigens used were TNP-B1-8 (▲), TNP-267.7 (△), TNP-N1G9 (●), TNP-S24-1 (○), and TNP-B1-48 (□). After 8 d, IgG1 anti-TNP titers in culture supernatants were determined by RIA. Arithmetic mean titers and standard deviations are given.

FIGURE 4. Enhancement of idiotype expression by N1G9-specific T helper cells in a secondary IgG1 anti-NP response: 1×10^6 Lyt-2⁻, N1G9-primed (closed columns) or control (adjuvant alone) lymph node T cells (hatched columns) were cultured with 4×10^6 T cell–depleted, NP-primed SC in the presence of 100 ng NIP-POL/ml in 1-ml triplicate cultures. As a control, N1G9-primed T cells were cultured with T cell–depleted, NP-primed SC in the absence of NIP-POL (open columns). 8 d later, titers of anti-NP and idiotope-bearing antibodies were determined by RIA. Geometric mean titers and standard deviations are given.

by isotype both in the carrier inhibition experiments and the experiments in Fig. 3.

Enhancement of Idiotype Expression at the Level of IgG1 by N1G9-specific T Helper Cells in a Secondary Anti-NP-Response. We next asked whether the N1G9-recognizing T cells would affect idiotype expression in anti-NP responses. T cell–depleted, NP-primed SC were cultured in vitro together with either N1G9- or CFA-primed T cells, in the presence of the TI-II antigen NIP-POL. A representative result is shown in Fig. 4. Anti-NP responses were induced both at the level of IgM and IgG1. They depended upon the presence of antigen and their size was unaffected by the N1G9-specific T cells. However, when the distribution of idiotypic determinants in the response was analyzed, the cells were found to exert a clear-cut effect: the expression of certain N1G9-specific idiotopes (Ac146, A39-40, and A6-24, identified as potential idiotypic targets in the TNP carrier experiments [Fig. 3]) was enhanced in their presence. Significantly, this enhancement was only seen in IgG1 but not IgM antibodies.

Discussion

The recognition by T cells of idiotype presented on IgG1 but not IgM was observed in this study first in the induction of the cells by immunization, second, at the level of carrier recognition in the anti-TNP response to TNP-coupled idiotype (an assay used earlier for the identification of idiotype-specific T cells by others [reviewed in 11, 12]), and third, in the enhancement of idiotype-bearing IgG1 antibodies in the secondary anti-NP response.

It is unlikely that this phenomenon is due to a collaboration of idiotype-specific T cells and T cells recognizing isotype or spontaneously producing B cell differentiation factors, since, in this case, the helper function of the idiotype-specific cells in the anti-TNP response should be inhibitable by antibody B1-8 as well as by N1G9, and this is not the case (Fig. 2). In addition, antibody B1-8 would be expected to induce helper activity with specificity for N1G9 and/or B1-8 as efficiently as antibody N1G9, and this is again not observed (Fig. 1 and unpublished data).

We therefore think that the helper T cells identified in our study react to idiotypic determinants in association with determinants of the heavy chain constant region. The structural basis of this complex specificity is unknown. An antiidiotypic antibody exhibiting a similarly restricted binding specificity has been described (13). One might also speculate that IgM and IgG antibodies are differently processed or that immunization with an antibody of the IgG class leads to the induction of IgG-specific Fc receptors on antiidiotypic T cells (14), resulting in a cell expressing idiotype- and isotype-specific receptors. Finally, we might mention that isotype restriction has been considered as an alternative to major histocompatibility complex (MHC) restriction and a potential general principle in T cell–mediated network control (for review see 1, 11).

We have decided not to go into a further analysis of the functional specificity of the T cell population described in this paper. Instead, we are attempting to isolate individual helper cell clones to study their fine specificity and heterogeneity in a straightforward way, as well as to establish whether they are MHC restricted, an issue of debate in the literature (reviewed in 11). Potentially, regulatory T cells of the kind described here might explain the observation that the enhancement and suppression of idiotype expression induced by antidiotypic antibody is most pronounced at the level of IgG, as compared with IgM, antibodies (1). This is supported by the fact that, in the present system, the regulatory cells enhance the expression of precisely the same idiotypic subset(s) of (IgG) anti-NP antibodies (Fig. 4) that we have previously shown to be preferentially enhanced or suppressed upon administration of antiidiotypic antibody (2). In the case of suppression, regulatory T cells are known to play a role (5). IgG-induced and -restricted antiidiotypic helpers might also be involved in the selection of antibodies in anamnestic responses.

Summary

Immunization with an IgG1 but not an IgM monoclonal anti-NP (4-hydroxy-3-nitrophenyl acetyl) antibody induced idiotype-recognizing T helper cells, although these two antibodies carry the same variable regions. The T cells appear to react to an idiotype on the IgG1 but not the IgM antibody. They selectively

enhance the expression of that idiotype in the IgG1 fraction of an in vitro anti-NP response.

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