T-cell proliferative response to hapten-modified self-immunoglobulins: Recognition of conjugate-specific determinants

(cell interaction/major histocompatibility complex/anti-idiotypic T cells)

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Communicated by Salome G. Waetsch, March 29, 1982

ABSTRACT (4-Hydroxy-3-nitrophenyl)acetyl (NP)-modified BALB/c immunoglobulins were used as the immunogen for induction of a proliferative response in BALB/c mice. As is true for responses to other soluble antigens, proliferation was dependent on Lyt-1 cells and histocompatible radioreistant accessory cells. Lyt-1 cells directed against NP-modified self immunoglobulin are specific for the immunizing hapten NP. However, they do not recognize hapten per se. Rather, they see complex determinants comprised of both the hapten NP and the immunoglobulin self-carrier. Distinct specificities were created by coupling the same hapten to different monoclonal BALB/c antibody molecules or by attaching the hapten to the immunoglobulin self-carrier via a spacer molecule. It is proposed that determinants created by attaching haptens to self-immunoglobulin molecules are similar to those recognized by anti-idiotypic T cells.

Individual T and B lymphocytes recognize unique antigenic determinants via specific receptor molecules on the cell surface. It is generally accepted that antigen receptors on B cells are conventional immunoglobulins (Igs) having the same antigen-binding specificity as secreted antibody molecules. By contrast, there exists a wide range of opinion regarding the precise chemical structure of the T-cell receptor. In particular, the extent to which antigen receptors on T cells resemble those on B cells has not been clearly resolved. On the one hand, there is considerable evidence that antigen receptors on T cells and B cells are different. For example, dual specificity [i.e., the need to recognize antigen in the context of self major histocompatibility complex (MHC) determinants] clearly distinguishes the T-cell receptor repertoire from that expressed by B cells (1–4). The observation that T and B cells predominantly recognize different epitopes on multidentator antigens also suggests that T and B cells use distinct receptors (5–7). Another battery of data indicates that antigen receptors on T cells and B cells are structurally similar. Antisera directed against variable regions on serum antibody molecules have been shown to have reactivity toward helper T cells (8, 9), suppressor T cells (10, 11), alloreactive T cells (12), and soluble T-cell products (13–16). These findings have been taken as evidence that the variable portions of T- and B-cell receptors are encoded by the same variable region genes.

To obtain additional information regarding the possible relationship between T- and B-cell receptors, it would be useful to study T-cell recognition of a simple well-defined antigen—a hapten. Hapten-specific T cells have been extensively described in the literature; the most commonly used strategy for induction of hapten-specific T-cell immunity has been to immunize with the hapten coupled to a self-carrier. One particularly well characterized system is the response to the hapten (4-hydroxy-3-nitrophenyl)acetyl (NP).

Anti-NP antibodies produced by C57BL/6 (B6) mice and other strains expressing the b allele at the IgH gene complex express a major crossreactive idiotype (the NPb idiotype) and carry a heteroclytic fine specificity marker; i.e., they have a higher affinity for structurally related haptenes such as (4-hydroxy-3-iodo-5 nitrophenyl)acetyl (NIP) than for the immunizing hapten, NP (17, 18). Several populations of NP-specific T cells have been analyzed for expression of these variable region markers. Effector T cells for NP-specific delayed-type hypersensitivity responses express the heteroclytic fine specificity marker (19). NP-specific suppressor cells bear NPb idiotype+ determinants on their surface (11). Soluble heteroclytic NPb idiotype+ receptor material has been isolated from a population of NP-sensitized T cells whose function has not yet been defined (13). By contrast, cytotoxic T lymphocytes directed against NP-coupled autologous cells (20) and NP-specific helper cells (21) express a nonheteroclytic phenotype. These observations are consistent with evidence for structurally distinct receptors on different T-cell subpopulations (22, 23).

The present study analyzes the fine specificity of T cells that proliferate in response to NP-modified self Ig. These Lyt-1-positive cells have specificity for the immunizing hapten, NP. However, they do not recognize hapten per se. Rather, they see conjugate-specific determinants—i.e., determinants contributed by both the hapten, NP, and the Ig self-carrier. Distinct specificities were created by coupling the same hapten to different self-Ig molecules or by attaching the hapten to the Ig self-carrier via a spacer molecule. The possibility is considered that determinants created by attaching haptenes to self-Ig molecules may resemble determinants recognized by anti-idiotypic T cells.

MATERIAL AND METHODS

Animals. BALB/cAn mice were progeny of animals obtained from the Kingstone facility of Charles River Breeding Laboratories. CB20 mice were derived from breeding stocks kindly provided by Michael Potter, National Cancer Institute, Bethesda, MD. BALB.B and BALB.K mice were generously provided by Maurice Zauderer, Columbia University, New York. Animals of either sex were used at 6–12 wk of age.

Antigens and Other Reagents. Normal mouse and rabbit Igs were purified by ammonium sulfate precipitation and chromatography on DEAE-cellulose. Affinity-purified S107 and

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Ar2.7 (a BALB/c anti-p-azophenylarsonate IgG1, κ-bearing hybridoma protein) were generously provided by Angela Gusti and Dale Yelton, respectively. Clarified ascites containing MOPC 104E was purchased from Litton Bionetics, and the 104E was further purified as 19S IgM by chromatography on Sephadex G-200. Chicken gamma globulins (fraction II) were purchased from Miles Laboratories. NP-OSU (succhinimide ester), NIP-OSU, NP-6-aminoacaproic acid (εAcp)-OSU, NIP-εAcp-OSU, and trinitrobenzene sulfonate were purchased from BioSearch, San Rafael, CA. NP and NIP derivatives were synthesized as described (24) except that succinimide esters rather than azides were used for conjugation. TNP derivatives were prepared according to the same protocol except that Igs were allowed to react with trinitrobenzenesulfonate for 10–20 min at room temperature. The average substitution was 15–20 hapten groups per Ig molecule. Preparation of fluorescein-conjugated Igs was carried out as described by Hudson and Hay (25) using fluorescein isothiocyanate from Baltimore Biological Laboratory, Cockeysville, MD; conjugates contained 10–15 mol of fluorescein per mol of protein. All hapten-modified Igs were dialyzed against 0.1 M KHCO₃ and then sterilized by passage through a 0.45-μm Milipore filter, and stored at 4°C.

Immunization. Hapten-self-Ig conjugates were emulsified in complete Freund’s adjuvant (CFA) (Difco). Fifty microliters containing 100 μg of antigen were injected subcutaneously at the base of the tail (26).

Cell Cultures. Lymph node proliferation assays were carried out by a slight modification of the method of Corradin et al. (26). Briefly, 7 days after immunization, inguinal and para-aortic lymph nodes were removed and teased into single cell suspension. Cells were placed into 96-well flat-bottomed Linbro microtiter plates at a density of 5 × 10⁵ cells in 200 μl of RPMI 1640 supplemented with 5% fetal calf serum/2 mM glutamine/15 mM Hepes, pH 7.2/1 mM sodium pyruvate/50 μM 2-mercaptoethanol/penicillin (100 units/ml)/streptomycin (100 μg/ml) per well. They were cultured for 3 days. Then, proliferation was assessed by a 16- to 18-hr exposure to 1 μCi of [³H]thymidine (1 Ci = 3.7 × 10¹² becquerels; Amersham). Results are expressed as means of triplicate cultures.

T-Cell Populations. Monoclonal anti-Thy-1.2 was generously provided by Malcolm Gefter, Massachusetts Institute of Technology, Cambridge, MA. Anti-Lyt-1.2 and anti-Lyt-2.2 antisera were the gift of Harvey Cantor, Sidney Farber Cancer Center, Boston, MA. Freshly frozen rabbit serum absorbed with mouse liver, spleen, thymus, and lymph node was used as a source of complement. Lymph node cells were incubated at room temperature at 5 × 10⁷/ml in the presence of anti-Thy 1.2 (1:10⁴), anti-Lyt 1.2 (1:40), anti-Lyt-2.2 (1:30), or normal mouse serum (1:30) diluted in balanced salt solution. After 30 min, the cells were spun out of the antisera, resuspended in complement (1:10 in balanced salt solution; 5 × 10⁶ cells/ml) and incubated for an additional 45 min at 37°C. Cells were then washed and this two-step treatment was repeated once again.

Short-term T-cell cultures were used as an enriched source of NP-BALB/c Ig-reactive T cells. NP-BALB/c Ig-immune lymph node cells (2 × 10⁷) were incubated in 5 ml of complete medium described above containing NP-BALB/c Ig at 100 μg/ml in Falcon flasks (3013, Falcon Labware). Seven days later, T cells were placed in fresh medium containing partially purified T-cell growth factor (Collaborative Research, Waltham, MA) and restimulated with antigen in the presence of a 50-fold excess of syngeneic irradiated spleen cells. The requirement for MHC-compatible accessory cells was tested 7 days after subculture.

Preparation of Irradiated Spleen Cells. Spleens from unprimed mice were teased into single cell suspension with forceps. Erythrocytes were lysed by incubation for 2 min at 37°C in 0.17 M ammonium chloride/10 mM Tris-HCl, pH 7.2. Spleen cells were then irradiated with 2,000 rads (1 rad = 0.01 gray) from a cesium source (Gammacell, Atomic Energy of Canada, 142 rad/min).

RESULTS

Required Participation of Lyt-1 Cells. BALB/c mice were immunized with 100 μg of NP-BALB/c Ig in complete Freund’s adjuvant subcutaneously at the base of the tail according to the method of Corradin et al. (26). Seven days later, inguinal and paraaortic lymph node cells were stimulated in vitro with the homologous antigen NP-BALB/c Ig. As shown in Fig. 1, proliferation was sensitive to treatment with anti-Thy-1.2 or anti-Lyt-1.2 but not anti-Lyt-2.2 and complement. Thus, Lyt-1-positive cells are required for the proliferative response to NP-modified self Ig.

Requirement for Histocompatible Antigen-Presenting Cells. In preliminary experiments, it was observed that nylon-purified T cells were unable to respond to NP-modified self Ig and that responsiveness was reconstituted by the addition of nonimmune irradiated syngeneic spleen cells (data not shown). These results indicated that proliferation was dependent on an interaction between Lyt-1 cells and antigen-presenting cells. Recent experiments of Bottomly and her collaborators suggest that T cells that recognize antigenic determinants on self-Ig molecules are not MHC restricted (27, 28). It was therefore of interest to learn whether Lyt-1 cells recognize NP-modified self Ig in association with self MHC products. Short-term cultures were used as an enriched source of NP-self Ig-reactive T cells. After 14 days of selective stimulation in vitro in the presence of antigen, alloreactivity was greatly depleted although not completely eliminated (Table 1). Under these conditions, there was a clear requirement for MHC-compatible radioresistant accessory cells.

![Fig. 1. Required participation of Lyt-1 cells. Various concentrations of NP-BALB/c Ig were added to cultures containing 5 × 10⁵ NP-BALB/c Ig-immune lymph node cells treated with normal mouse serum (○), αLyt-2.2 (●), αThy1.2 (○), or αLyt-1.2 (■) plus complement.](image-url)
for antigen-dependent proliferation. Thus, cells that participate in the proliferative response to NP-modified self Ig have the same characteristics as cells that participate in proliferative responses to other soluble antigens.

Recognition of Conjugate-Specific Determinants. Based on the assumption that T cells are tolerant to the self-Ig carrier, it was possible that responding Lyt-1 cells were directed against the hapten NP. Consistent with this idea, there was no cross-reactivity between NP-BALB/c Ig and BALB/c Ig modified by a different hapten, such as fluorescein (Fig. 2). However, there was also no crossreactivity between NP-BALB/c Ig and NP coupled to a heterologous carrier, such as chicken gamma globulin (Fig. 2). Thus, Lyt-1 cells directed against NP-modified self Ig see more than the hapten moiety itself. Rather, these Lyt-1 cells recognize conjugate-specific determinants—i.e., determinants contributed by both the hapten and the Ig self-carrier.

Discrimination Among Different NP-Modified Self Igs. The contribution of the Ig self-carrier was further evaluated by analyzing reactivity toward different NP-modified self Igs. As shown in Fig. 3, there was no crossreaction between NP-BALB/c Ig made from a pool of DEAE-purified serum IgG and NP conjugates prepared with different monoclonal BALB/c Igs, such as NP-104E (IgM, λ) or NP-S107 (IgA, κ). Moreover, the absence of a response to NP-104E or NP-S107 is not due to lack of immunogenicity with these conjugates because, as shown in Fig. 4, when BALB/c mice were immunized against NP-104E and challenged in vitro with the homologous conjugate NP-104E, there was a vigorous proliferative response. Considering that the 104E was not affinity purified but simply taken as the excluded volume on a Sephadex G-200 column, the partial crossreaction between NP-104E and NP-Ar2.7 seen in Fig. 4A (Ar2.7 is a BALB/c IgG1, κ-bearing monoclonal anti-p-azophenylarsonate antibody that was affinity purified) is probably due to a low level of contaminating IgG1 in the 104E. This interpretation is supported by the lack of crossreactivity in the reciprocal experiment in Fig. 4B. Taken together, data presented in Figs. 3 and 4 show that Lyt-1 cells recognize distinct specificities on different NP-modified self Igs.

Fine Specificity of the Proliferative Response to NP-Modified Self Ig. Considering that responding Lyt-1 cells recognized determinants contributed in part by the hapten NP, it was of interest to learn whether these T cells express the heteroclytic fine specificity marker associated with B-cell anti-NP responses. Fine specificity of the proliferative response to NP-modified self Ig was therefore assessed in CB20 mice, a strain congenic with BALB/c but expressing the IgH-1β allotype of B6. As shown in Fig. 5, Lyt-1 cells from CB20 mice immunized against NP-modified B6 Ig were highly responsive on secondary challenge with the homologous conjugate NP-B6 Ig. There was also a partial crossreaction on the structurally related conjugate NP-B6 Ig. However, the magnitude of this response was not significantly different from that of BALB/c Lyt-1 cells (Fig. 5). (BALB/c mice express the a allele at the IgH gene complex and do not express the NPβ idotype). Thus, responding Lyt-1 cells express a nonheteroclytic phenotype. Moreover, Lyt-1 cells directed against NP-modified self Ig did not have any measurable reactivity toward NP-eAcp-self Ig—i.e., NP coupled to self Ig via a caproic acid spacer (Fig. 5). This result supports the

![Fig. 2. Recognition of conjugate-specific determinants. Various concentrations of NP-BALB/c Ig (○), fluorescein-BALB/c Ig (●), or NP-chicken gamma globulins (□) were added to cultures containing 5 × 10⁶ NP-BALB/c Ig-immune lymph node cells.](image)

![Fig. 3. Discrimination among different NP-modified self Igs. Various concentrations of NP-BALB/c Ig (○), NP-104E (●), NP-S107 (□), or NP-rabbit Igs (■) were added to cultures containing 5 × 10⁶ NP-BALB/c Ig-immune lymph node cells.](image)
DISCUSSION

The studies described in this report were initiated in an attempt to generate continuously growing T-cell lines that have reactivity toward the hapten NP. Immunization with hapten coupled to self Ig has been used previously as a strategy for induction of hapten-specific helper (9, 29) and suppressor (10, 30) cells. By analogy, it seemed reasonable to assume that T cells that proliferate in response to NP-modified self Ig would be directed against the hapten NP. However, the data presented above indicate that responding Lyt-1 cells recognize conjugate-specific determinants. Although somewhat surprising, this result is consistent with many previous descriptions of T-cell receptor specificity. Early experiments in guinea pigs by Benacerraf and Paul and co-workers (31, 32) demonstrated that delayed hypersensitivity reactions and proliferative responses to conjugates of guinea pig albumin were specific for both the immunizing hapten and the carrier. Similarly, Janeway (33) reported that T cells that proliferate in response to dinitrophenyl-modified mycobacteria recognize dinitrophenyl coupled to some but not all carriers. Thus, it has been known for some time that T cells recognize complex determinants created by the combination of a particular hapten coupled to a particular carrier molecule. The conclusion that T-cell receptor specificity is quite different from that of antibody is also supported by more recent experiments showing that T cells and antibodies are predominantly directed against different epitopes on multideterminant antigens (6, 7).

These results suggest that MHC-restricted T cells are specific for self plus X, while B cells recognize Y, where X and Y are nonoverlapping sets of determinants. However, individual antigenic determinants for MHC-restricted T cells have not been clearly defined. The idea that B cells recognize X, while T cells recognize self plus X has persisted in this climate of ambiguity.

The antigen most frequently claimed in the past to be capable of eliciting hapten-specific help was a hapten-self Ig conjugate (9, 29). I have analyzed the fine specificity of Lyt-1 cells that proliferate in response to NP-modified self Ig. These T cells were specific for the immunizing hapten, NP, in that they did not recognize the Ig self-carrier modified by different haptons. However, NP coupled to self Ig via a caproic acid spacer molecule was recognized as a distinct antigenic specificity, and NP conjugates prepared with different self Igs were not crossreactive. Thus, responding Lyt-1 cells were not directed against the hapten NP. Rather, these T cells, like those that respond to hapten-self albumin conjugates, show conjugate specificity; i.e., they recognize determinants contributed by both the hapten NP and the Ig self-carrier. How can these data be reconciled with previous work describing hapten-specific helper T cells? Considering that, in the present study, Lyt-1 cells were not tested functionally, one possibility is that Lyt-1 cells that proliferate in response to NP-self Ig conjugates may not help B cells. However, this would be an exception to the general rule since MHC-restricted Lyt-1 cells that proliferate in response to other soluble antigens have helper activity for B cells (34–36). An alternative explanation is that the Lyt-1 cells described here have helper activity, but not all hapten-self Ig-reactive helper cells express the same type of receptor specificity. This proposal is consistent with data indicating that there are two distinct types of helper cells that act synergistically for the induction of optimal antibody responses (27, 29, 37–40). One of these is MHC restricted and has specificity for the immunizing carrier, properties similar to those demonstrated for NP-self Ig-reactive Lyt-1 cells described here. Bottomly and co-workers (27, 29) have characterized a second antigen-specific helper cell that is not MHC-restricted (27, 28). Perhaps hapten-specific helper cells described previously belong to this second category of
helper cells. In the absence of information regarding their MHC restriction specificity, it is difficult to evaluate this hypothesis. Finally, it is also possible that so-called hapten-specific helper cells described in the past were, in fact, directed toward conjugate-specific determinants. As shown here, conjugate-specific Lyt-1 cells recognize determinants contributed in part by the immunizing hapten. Thus, a lack of reactivity towards self Ig modified by an irrelevant hapten is not necessarily indicative of hapten specificity. The idea that hapten-modified self Ig-reactive helper cells are directed toward the hapten also derives from the finding of shared idiotypes on helper cells and anti-hapten antibodies (9). However, antibodies to different epitopes on multidentate antigens sometimes express cross-reactive idiotypes (41–44). Thus, conjugate-specific helper cells and anti-hapten antibodies directed against distinct determinants on hapten-self-Ig conjugates may well bear common idiotypes. In short, one cannot be certain that previously characterized hapten-modified self Ig-reactive helper cells were truly hapten specific.

Whether or not T and B cells recognize the same epitopes on hapten-self-Ig conjugates has important implications for understanding the specificity of anti-idiotypeic T cells. It is known that anti-idiotypeic T cells are induced after immunization with idiotype (9, 45, 46). Auto-anti-idiotypeic T cells are thought to play a key role in regulating network interactions. Whether anti-idiotypeic T and B cells recognize the same or different idiotypes is unknown. A strong argument can be made that coupling the hapten NP to arginine and lysine residues on self-Ig molecules mimics a naturally occurring mutation in which there is a replacement of a charged hydrophilic amino acid by a neutral hydrophobic residue. According to this idea, conjugate-specific determinants on hapten-modified self Ig resemble new determinants on variant antibody molecules. The particular IgGs used in this study have extremely different structures, and nitrophenylated molecules were not crossreactive. Discrimination among different NP-modified self IgGs cannot simply be due to recognition of known serologically defined specificities because attempts to elicit responses to unmodified self IgGs have so far been unsuccessful (unpublished observations). The assumption that similar specificities may be created by coupling the same hapten to closely related IgGs can be tested in future experiments. In sum, the Lyt-1 proliferative response to hapten-modified self Ig should provide a useful model for studying T-cell recognition of self and nonself Ig.

I thank Drs. C. Bona, R. Schwartz, and M. Zauderer for valuable discussion and critical review of this manuscript. This work was supported in part by National Institutes of Health Grant AI-19047.