Brief Definitive Report

REGULATORY IDIOTOPES

Induction of Idiotype-recognizing Helper T Cells by Free Light and Heavy Chains

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Since the structure of the T cell receptor appears to be unrelated to a conventional immunoglobulin (1–5), the immunochemical and cellular results showing cross-reactivity between B and T cell surface receptors need to be reinterpreted (6–8). If T cell receptors were encoded by the same V_H gene pool used by B cell receptors and antibodies, the specificity of T cell recognition would be very similar to that of B cells. However, several lines of evidence show that T cells recognize their target differently than B cells (9–12). An additional difference between B and T cells is that the T cell repertoire is "educated" during its development through exposure to the microenvironment of the thymus (13).

For the purpose of having a hypothesis consistent with most data on T cell function and specificity, including the data of this report, we assume that the T cell repertoire is educated by contact with B cell idiotopes. This prepares the T cells to (*a*) interact with the B cells in the control of immune responses and (*b*) to recognize nominal antigen because of internal antigen imaging by B cell idiotypes (14). The primary idiotope complementarity of B and T cell receptors implies that B cell idiotopes are recognized in an absolute sense regardless of their association with antigen specificity. Thus, idiotopes of individual immunoglobulin polypeptides (heavy and light chains) must be considered primary targets for T cells.

Here we report that the induction of T helper (Th) cell circuits is achieved by priming with light or heavy chains. Th_2 cells, which are specific for the shared antiphosphorylcholine (anti-PC) idiotopes of TEPC-15 (T15) and MOPC-167 (M167) are induced by the free heavy chain of T15 or 167; Th_1 cells, which stimulate Th_2, can be induced by priming with the free light chain of a monoclonal anti-T15 antibody.

Materials and Methods

Mice and Immunizations. NBF_1 male mice were obtained from Dominion Labs, Dublin, VA. Athymic, nu/nu BALB/c mice were obtained from our own breeding colony. NBF_1 males to be used as T15-, M167-, or F6-3-primed donors received 100 μg of the protein in complete Freund's adjuvant (CFA) intraperitoneally; 4 wk later, they received 50 μg of

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the protein in incomplete Freund's adjuvant (IFA) intraperitoneally, and were used 4 wk later. Other NBF_1 males received 50 μg of heavy or light chains of T15, M167, or F6-3 in CFA intraperitoneally 8 wk before use; 4 wk before use, the mice received 50 μg of the respective heavy or light chain in IFA intraperitoneally.

Myeloma and Hybridoma Proteins. The plasmacytomas T15, M167, and MOPC-460 (M460) were obtained from Dr. M. Potter, National Cancer Institute, Bethesda, MD, and were affinity-purified from ascitic fluid on a PC-Sepharose column. The anti-T15 hybridoma F6-3 was prepared in our lab as described (15).

Antigens. The trinitrophenylated antigens were prepared by reacting 2,4,6-trinitrobenzene sulfonic acid (TNBS) (Sigma Chemical Co., St. Louis, MO) with T15, M167, or M460 that had had their binding sites blocked by 10^{-2} M PC-chloride (Sigma Chemical Co.) or 0.1 M dinitrophenyl glycine (Sigma Chemical Co.). The antigens were separated from free trinitrophenyl (TNP) by dialysis against borate-buffered saline.

Separation of Immunoglobulin Heavy and Light Chains. Isolated T15, M167, or F6-3 heavy and light chains were prepared from affinity-purified proteins that had been mildly reduced and alkylated with 0.2 M dithiothreitol (Calbiochem-Behring Corp., San Diego, CA) and 0.44 M iodoacetamide (Eastman Kodak Co., Rochester, NY). The reduced and alkylated proteins were dialyzed overnight in 1 M propionic acid and the chains then separated by passage over a G-100 Sepharose column (Pharmacia Inc., Piscataway, NJ). The purity of the heavy and light chains was analyzed on sodium dodecyl sulfate (SDS) polyacrylamide electrophoresis by the method of Steck et al. (16).

Donor T Helper Cell Preparation. The donor cell population was prepared by passage of spleen cells over a nylon wool column. Lyt-2^+ T cells were removed by treatment of the nonadherent cell population with anti-Lyt-2 hybridoma antibody 53-6.72 (American Type Culture Collection, Rockville, MD) and low toxicity rabbit complement (Accurate Chemical and Scientific Corp., Westbury, NY). The purity of the Lyt-2^- population was tested as described (12).

Fragment Cultures and Enzyme-linked Immunoabsorbent Assay (ELISA). The splenic fragment culture system for carrying out limiting dilutions of T cells has been described (12, 17). Briefly, graded numbers of Lyt-2^- T cells were transferred into nu/nu BALB/c recipients. 24 h later, the recipient spleens were removed and chopped into 1-mm cubes that were cultured separately in sterile 96-well culture plates (Costar, Data Packaging, Cambridge, MA) in Dulbecco's modified Eagle medium (DME) (Gibco Laboratories, Grand Island, NY) enriched with 10% agammaglobulin horse serum (Gibco Laboratories) plus TNP_7-T15 or TNP_{12}-M167 at a concentration of 10^{-8} M TNP. After 3 d of culturing, the antigen was removed and fresh DME added. Culture fluids were collected for analysis every 3 d thereafter, and the ELISA (18) was used to detect anti-TNP antibody as previously described (12).

Results and Discussion

The specificity through which "idiotype-like" and "antiidiotype-like" T cells interact with other cells in the regulation of the immune repertoire remains of great interest. To study the T cell specificity, we are using a T helper cell network that can induce T15/M167 idiotype-recognizing T cells. Direct and indirect priming schemes using T15 and M167 idiotypes, PC antigen, and anti-T15 antibodies are effective (12, 19, 20). The T cell population stimulated by these manipulations recognizes an idiotypic determinant shared by both T15 and M167. Thus, to define the shared determinant able to activate the idiotype-recognizing T cells, we further dissected the idiotype and antiidiotype induction pathways by using free heavy and light chains of T15, M167, and of monoclonal anti-T15 (F6-3) to specifically prime the T helper cells in vivo. The specific antigenicity obtained when T cells were primed with whole immunoglobulin or separated immunoglobulin chains was compared. To preserve as much as possible the native structure of free chains, complete reduction and alkylation of the

TABLE I

Priming of Idiotope-recognizing T Helper Cells with T15 Heavy and Light Chains

NBF_1 male donor T cell priming*	Number of Th cells transferred‡	In vitro antigen§	Percent positive anti-TNP cultures‖
—	10^6	TNP-T15	1
T15	10^6	TNP-T15	24
T15	10^6	TNP-M167	26
T15 H chain	5×10^5	TNP-T15	18
	10^6		32
	10^6	TNP-M167	36
	5×10^5		24
	10^6	TNP-M460	6
T15 L chain	5×10^5	TNP-T15	10
	10^6		8
	5×10^5	TNP-M167	10
	10^6		6

* NBF_1 male donor mice were immunized with 50 μg of affinity-purified T15 in CFA, or with 50 μg of separated T15 heavy chain or light chain in CFA 8 wk before use; 4 wk later, the mice were given a second immunization of 50 μg of T15, T15 heavy chain, or T15 light chain in IFA, and were used 4 wk later as spleen cell donors.

‡ Th cells were prepared by passage over nylon wool columns and subsequent treatment with anti-Ly-2 and rabbit complement. Graded doses of Lyt-2^- T cells were injected intravenously into nu/nu BALB/c mice. 4 h later, splenic fragment cultures were prepared.

§ The fragment cultures were immunized in vitro with TNP_7-T15, TNP_{12}-M167, or TNP_6-M460 at 10^{-6} M TNP.

‖ 48–96 splenic fragment cultures were assayed for anti-TNP activity on days 9 and 12.

TABLE II

Priming of Idiotope-recognizing T Helper Cells with M167 Heavy and Light Chains

NBF_1 male donor T cell priming*	Number of Th cells transferred‡	In vitro antigen§	Percent positive anti-TNP cultures‖
—	10^6	TNP-M167	0
M167	10^6	TNP-M167	36
	10^6	TNP-T15	24
M167 H chain	5×10^5	TNP-M167	34
	10^6		18
	5×10^5	TNP-T15	23
	10^6		4
	10^6	TNP-M460	0
M167 L chain	5×10^5	TNP-M167	8
	10^6		12
	5×10^5	TNP-T5	8
	10^6		0

* Donor T cells were primed with M167, M167 heavy chain, or M167 light chain as in Table I.

‡ T helper cells were prepared and transferred as in Table I.

§ Splenic fragment cultures were immunized as in Table I.

‖ 48–96 fragment cultures were assayed by ELISA as in Table I.

proteins was avoided and the chains were separated on a G100 column in propionic acid.

The results shown in Tables I and II demonstrate two contrasting findings: the high responses generated towards both TNP-T15 and TNP-M167 when

TABLE III

Induction of Idiotope-specific T Helper with Antiidiotype Heavy and Light Chains

Donor T helper cell priming*	Number of T cells transferred‡	In vitro antigen§	Percent positive anti-TNP positive cultures‖
—	None	TNP-T15	0
—	None	TNP-M167	0
F6-3	5×10^5	TNP-T15	14
	10^6		32
	5×10^5	TNP-M167	24
	10^6		46
F6-3 H chain	5×10^5	TNP-T15	2
	10^6		2
	5×10^5	TNP-M167	2
	10^6		12
F6-3 L chain	5×10^5	TNP-T15	14
	10^6		22
	5×10^5	TNP-M167	22
	10^6		34

* Donor NBF_1 males were immunized with monoclonal anti-T15 (F6-3) according to the protocol in Table I.

‡ $Ly\text{-}2^-$ T cells were prepared as in Table I.

§ TNP-T15 or TNP-M167 were used as in vitro antigens as in Table I.

‖ 48–96 splenic foci were assayed for anti-TNP antibody as described in Table I.

T15, M167, or their respective heavy chains are used to prime T cells, and the low response induced by T15 or M167 light chains. To ensure that the priming by free T15 or M167 heavy chains was not against the constant region of IgA allotypes, the response to a control antigen of the same immunoglobulin class was monitored. As seen in Tables I and II, the B cell response to TNP-M460 is not significant. These data localize the important epitopes for T cell induction on the heavy chains of T15 and M167. This finding indicates that the epitope recognized by the T helper cells is not dependent on the 7S immunoglobulin conformation, but rather on heavy chain determinants. This heavy chain expitope specificity of T helper cells accords with earlier observations that only T15 and M167 heavy chains and not light chains can inhibit the action of the idiotype-recognizing cells. Further, since both T15 and M167 can prime the helper cells equally well, the immunizing epitope would have to be shared by the two idiotypes; T15 and M167 have very similar heavy chains, differing by only a few amino acids, but their light chains are very different (21).

In a previous report, we described the $Th_1 \rightarrow Th_2$ cellular circuit that operates when the idiotype-recognizing Th_2 is induced by priming with a monoclonal anti-T15 idiotype, F6-3 (12). To determine which epitopes on the antiidiotope are responsible for stimulating the Th_1 cell, we examined the antigenicity of F6-3 antibody and of its separated heavy and light chains. Table III details the responses to TNP-T15 and TNP-M167. Unlike direct priming with T15 or M167, F6-3 light chain exhibits as much antigenicity as whole F6-3 protein, while the heavy chain of F6-3 induces only a background response to the two antigens.

This result was unexpected in light of our previous findings, in which the heavy chain is the part of the antibody most responsible for inhibition and priming of idiotype-defined cells (this report and 22). However, idiotype-specific

priming with light chain was demonstrated in the 315 system by Jorgensen and Hannestad (11), and anti-light chain sera has been implicated in the inhibition of hypersensitivity and transplantation reactions (23). The explanations for these findings may lie in Jerne's concept of the internal image of an antigen (14). The internal image can be described as a structure that sterically resembles the original antigen. A specific antiantibody, Ab2β, may react with Ab1 because Ab2β sterically resembles the immunizing antigen. In the situation described here, the separated light chains of the monoclonal Ab2, F6-3, may form stable dimers that express the internal image of the PC antigen, which we have now shown is also able to induce the Th_2 cell via the Th_1 intermediate. Our data indicate that the primary targets of recognition for T cells are specific B cell idiotopes localized on either heavy or light chains. T cells are educated during thymic maturation to recognize B cell idiotopes that sterically mimic antigen and thus can expand T cells. In this model, regulatory T cells (12, 24) preferentially recognize idiotopes that mimic nominal antigens. Further experiments including binding and crystallographic studies on idiotype-antiidiotype complexes will be needed to substantiate this idea.

Summary

Previously, we have demonstrated the induction of T helper cells that recognize idiotype by antigen (19), idiotype (20), and antiidiotype (12). The T cell population has been characterized and found to recognize both the T15 and M167 myeloma proteins, which share PC binding specificity but differ in idiotypic specificities. In the present work, we used isolated heavy and light chains of T15 and M167 to generate T helper cells, and examined the response to trinitrophenyl (TNP)-T15 and TNP-M167. We found that the heavy chains induced a dose-dependent response to TNP-T15 and TNP-M167, while the light chain priming was ineffective. When isolated chains of a monoclonal anti-T15 antibody (F6-3) were used to induce idiotype-recognizing T cells, only the F6-3 light chains generated T cell help for TNP-T15 and TNP-M167. Evidently, the idiotypic determinant that is recognized by the T cells is not dependent upon the conformation of combined heavy and light chains. These data show that the Th_2 helper cells for the T15/M167 idiotopes are induced by free heavy chains of T15 and M167; the Th_1 T cells that interact with the Th_2 population, however, can be triggered by free light chains of an antiidiotypic hybridoma antibody. These provocative findings suggest a new model for the T helper cell network.

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