SENDAI VIRUS–SPECIFIC T CELL CLONES

V. Induction of a Virus-specific Response by Antiidiotypic Antibodies Directed Against a T Helper Cell Clone

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The immune response is thought to be regulated by a delicate balance of idiotypic-antiidiotypic interactions (1). Introduction of antigen leads to clonal expansion of idiotypic B cells that secrete antibodies. These antibodies in turn stimulate an antiidiotypic immune response that may suppress the initial idiotypic B cell response. The antiidiotypic antibody may mimic the antigen and thus (even in a naive host) initiate an antigen-specific (idiotypic) B cell response (2, 3). In addition, antibody-induced antiidiotypic antibodies have been shown to induce (or suppress) activation of T cells (4–6). T cells carry idiotypic receptors structurally distinct from immunoglobulin molecules which compose the idiotype receptors of B cells. B cells have high affinity for free antigen while T lymphocytes have low affinity for antigen and require, in addition, recognition of products encoded for by genes of the major histocompatibility complex (MHC) (7–9). Whether T cells recognize a neoantigen composed of foreign antigen and MHC-encoded molecules using a single receptor (altered-self hypothesis) or recognize both antigens separately with two receptors (dual receptor hypothesis) is unknown.

Several groups have recently described antibodies against cloned T cell lines/hybridomas (10–14). These antibodies bind with high specificity to the T cell clones used for immunization and (depending on the system) either stimulate or suppress the antigen-specific immune response of these clones in vitro. In addition, these antibodies immunoprecipitate a 90,000 dalton dimer that can be separated under reducing conditions into two distinct monomers. This molecule presumably represents the T cell receptor.

Our group has recently developed an antiidiotypic antibody directed against a Sendai virus–specific T helper cell clone. We used this antiidiotypic antibody to induce a Sendai virus–specific immune response in vivo.

Material and Methods

Mice. Female B10.D2 (H-2^d), DBA/2 (H-2^d), BALB/c (H-2^d), C57BL/6 (H-2^b), AKR (H-2^k), and AJ (H-2^a) mice were purchased from The Jackson Laboratories, Bar Harbor, ME and used at the age of 6–10 wk.

Viruses.   Sendai virus and influenza A/PR8 virus were grown in embryonated eggs as described previously (15). Sendai virus was purified by centrifugation through a 10–40% sucrose gradient (90 min at 15,000 rpm, Beckman rotor SW 55 [Beckman Instruments, Inc., Fullerton, CA]). Sendai virus was inactivated by a 10-min exposure to ultraviolet (UV) light.

Generation of an Antiidiotypic B Cell Hybridoma.   B10.D2 mice were immunized over a period of 6 mo in 14-d intervals with a Sendai virus–specific T helper cell clone (designated 2H3.E8; reference 16). Splenocytes were harvested 7 d after the last immunization and fused with NSI-1-Ag4-1 myeloma cells (17). Cells that secreted an immunoglobulin which bound specifically to the clone used for immunization were subcloned. One subclone (1B4.E6) was expanded and used for further experiments. Supernatants of 1B4.E6 were precipitated three times with a saturated ammonium sulfate solution and reconstituted to a protein concentration of 20 mg/ml. The antibody was classified as an IgM molecule by radial immunodiffusion. To exclude the possibility that 1B4.E6 recognized a Sendai virus antigen rather than a T cell specific–antigen, concentrated supernatant was tested for antiviral activity by an enzyme-linked immunosorbent assay (ELISA) as well as by hemagglutination inhibition assay (HIA) (15). Supernatants were negative in both assays for anti–Sendai virus antibodies.

Mediation of a Delayed-type Hypersensitivity (DTH) Response.   Mice were immunized intraperitoneally either once with UV light–inactivated Sendai virus (SVuv) or twice within 24 h with 200 μl of 1B4.E6 supernatant. 6–7 d later, mice were sacrificed and splenocytes were prepared and injected intravenously into naive recipient mice (0.8–1 × 10^8 cells/mouse) that were challenged immediately afterwards with 10^3 hemagglutinating units (HAU) infectious Sendai virus into the left footpad. Control mice received virus only or virus and nonimmune splenocytes (18). Footpad thickness was measured 4, 24, and 48 h later. The percent increase in footpad thickness was calculated using the formula: [(Thickness of left footpad) − (thickness of right footpad)/thickness of right footpad] × 100.

Histology.   Footpads were fixed, embedded in paraffin, stained with hematoxylin and eosin, and viewed for cellular infiltrates under light microscopy.

Results

We used an antiidiotypic antibody to induce an antiviral immune response in vivo. This antibody was generated by multiple immunizations of B10.D2 mice with a syngeneic Sendai virus–specific T cell clone. This clone has been identified as an Lyt-1$^-$2$^-$ T helper cell clone, which proliferates and secretes interleukin 2 (IL-2) upon recognition of Sendai virus presented by H-2I region–compatible stimulator cells (16). This antiidiotypic antibody (designated 1B4.E6) was produced by the fusion of spleen cells from immunized mice with NS-1 myeloma cells. It specifically bound to the clone used for immunization but failed to bind to T cells from unimmunized mice or T cells directed against an unrelated antigen (19).

Antiidiotypic antibodies directed against idiotypic antibodies (B cell receptors) have been shown to induce a T cell response in the absence of antigen (2). To test whether the 1B4.E6 antibody could induce a T cell–mediated immune response, splenocytes of Sendai virus– or 1B4.E6-immune mice were tested in an adoptive transfer system for mediation of a DTH response (Fig. 1) (18). Mice were inoculated (intraperitoneally) with 1B4.E6 supernatant (200 μl twice within 24 h) or once with SVuv. 7 d later these immunized mice were sacrificed and 0.8–10 × 10^7 spleen cells were intravenously injected into naive recipient mice that were then challenged with 10^3 HAU of Sendai virus in the left footpad. All tested mouse strains mediated, upon injection of Sendai virus– or 1B4.E6-immune syngeneic splenocytes and challenge with Sendai virus, a local inflam-
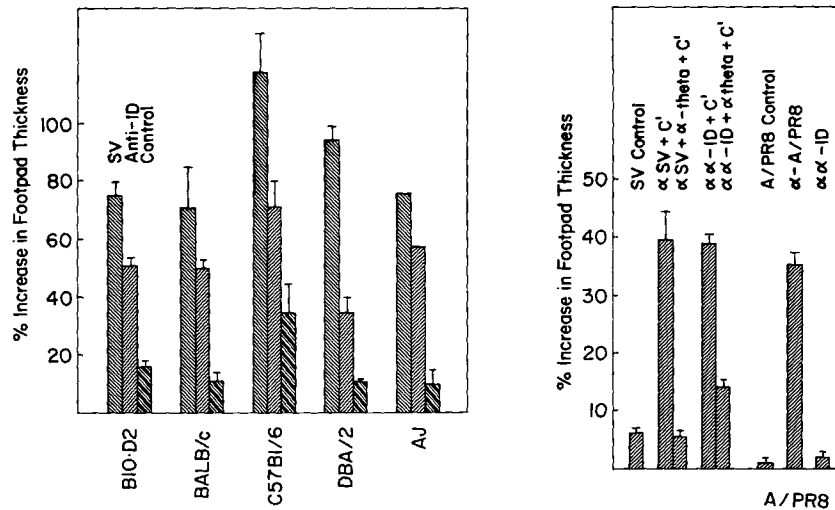
FIGURE 1   FIGURE 2

FIGURE 1. Induction of a DTH response with antiidiotypic antibody. Mice were injected with 10^3 HAU Sendai virus (SV) or antiidiotypic antibody (anti-Id). 1 wk later, splenocytes were transferred into naive recipient mice which were then challenged with Sendai virus into the left footpad. Control mice received virus only. Footpad thickness was measured 48 h later. FIGURE 2. Antigen specificity of induction of immunity with 1B4.E6. B10.D2 mice were injected with Sendai virus (SV), antiidiotypic antibody, or influenza A/PR8 virus (A/PR8). 1 wk later, SV and antiidiotype-immune splenocytes were treated with anti-theta antibody (monoclonal anti-Thy-1.2; New England Nuclear, Boston, MA) and complement (low-tox rabbit complement; Cedarlane) or complement only. Cells were transferred into recipient mice which were then challenged with the appropriate virus. Control mice received virus only. Footpad thickness was measured 48 h later.

matory response that histologically (infiltrate of monocytes) resembled a DTH response. No significant increase of footpad thickness was observed 4 h after the transfer of immune splenocytes. Maximal footpad swelling developed 24–48 h after transfer and then declined rapidly. The response was antigen-specific and mediated by T cells (Fig. 2).

We and others (20) have found that virus-stimulated DTH responses are MHC restricted in their effector phase (i.e., T cells from immune mice will not transfer immunity to MHC-incompatible animals). Unlike the Sendai virus–induced DTH response, the response induced by the antiidiotypic antibody showed a remarkable lack of H-2 restriction; i.e., in five of five experiments, 1B4.E6-immune splenocytes of H-2^b or H-2^k origin mediated a DTH response in H-2^d recipient mice (Table I). In two of five experiments, H-2^d donor cells elicited a response in H-2^b mice (experiment 1, Table I). In the other three experiments, H-2^d responder cells did not mediate a response in the allogeneic host (experiment 2, Table I is representative of these experiments). Cells transferred from H2^k donors immunized with 1B4.E6 had a similar lack of H-2 restriction (experiment 3, Table I). In all cases T cells induced by Sendai virus had absolute H-2 restriction and the transfer of allogeneic cells from nonimmune donors did not induce a DTH response (experiment 1). The degree of H-2 restriction seen with antiidiotype-induced cells varied depending upon the magnitude of the response. In all cases when a large response was achieved, the Sendai virus–induced cells

remained restricted in their ability to induce a DTH response while those sensitized with antiidiotype were not (Table I).

Discussion

In the present study we used an antiidiotypic antibody to induce an antiviral immune response in vivo. This antibody was generated by multiple immunizations of B10.D2 mice with a syngeneic Sendai virus–specific T helper cell clone (16) and the fusion of immune splenocytes with a myeloma cell line. It was characterized by its ability to bind to the T cell clone used for induction as well as to a high percentage of Sendai virus–specific T cell lines (19). The antibody failed to bind to naive T cells or to T cells directed against an unrelated antigen (such as a hapten or reovirus) and thus fulfilled the requirements for an antiidiotypic reagent (19). In spite of multiple immunizations over a long period of time (6 mo) this antiidiotypic antibody belonged to the IgM class.

The antiidiotypic antibody was capable of stimulating a T cell response, as shown by its ability to induce an antigen-specific DTH response. Stimulation of a DTH response by antibody-defined antiidiotypic antibodies has been described earlier (5, 21). Antiidiotypic antisera directed against alloantigens have been shown to stimulate proliferative T cells as well as cytolytic T cells (22). Antibodies directed against cloned T cells have been shown to either suppress or stimulate an immune response (11–14). In one system cloned T cells could be activated by clone-specific antisera in the absence of any accessory cells provided that exogenous lymphokines were added (12). These clones normally responded to antigen in the context of Ia molecules. These data can be interpreted either by a single T cell receptor model, assuming that the antibody recognizes and mimics an

TABLE I
H-2 Restriction of the Antiidiotype-induced DTH Response

Experiment	Donor mice	Immunization of donor mice	Percent increase in footpad thickness ± SD	
			B10.D2 (H-2^d)	C57BL/6 (H-2^b)
1	None	None	10 ± 9	6 ± 5
	B10.D2	None	24 ± 2	5 ± 5
		Sendai virus	61 ± 3	8 ± 5
		1B4.E6	53 ± 5	21 ± 3
	C57BL/6	None	1 ± 0	9 ± 4
		Sendai virus	10 ± 2	31 ± 4
		1B4.E6	43 ± 8	45 ± 9
2	None	None	5 ± 1	0 ± 3
	B10.D2	Sendai virus	22 ± 1	4 ± 1
		1B4.E6	26 ± 4	5 ± 3
	C57BL/6	Sendai virus	2 ± 2	23 ± 1
		1B4.E6	32 ± 6	31 ± 3
			B10.D2 (H2^d)	AKR (H2^k)
3	None	None	25 ± 3	21 ± 1
	AKR	Sendai virus	26 ± 1	86 ± 18
		1B4.E6	48 ± 3	86 ± 12

Groups of B10.D2, C57BL/6, or AKR mice were immunized with UV light–inactivated Sendai virus or 1B4.E6. Seven days later splenocytes of immune or naive mice were transferred into recipient mice. All mice were challenged with Sendai virus into the left footpad. Footpad thickness was measured 48 h later.

antigen/Ia complex, or by a dual receptor model that would imply that the affinity of the antiidiotypic antibody for the T cell receptor is higher than the affinity of conventional antigen, which only stimulates in association with H-2. In a two receptor model one would hypothesize that high affinity binding to one receptor is sufficient to trigger a response. In our system the affinity of the antiidiotypic antibody for T cells is most certainly higher than the affinity of antigen; i.e., the antiidiotypic antibody binds easily to T cells while soluble viral antigen does not. It is thus conceivable that the antibody can stimulate T cells in the absence of H-2 molecules (preliminary data indicate that, similar to the finding described by Kaye et al. [12], the 1B4.E6 antibody stimulates proliferation of the 2H3.E8 T cell clones in the absence of stimulator cells). But this would still not explain why T cells that have been induced with the antiidiotypic antibody show less H-2 restriction at the level of the effector phase than T cells stimulated by viral antigen. We can only speculate that the antiidiotypic antibody might stimulate a rare subset of T precursor lymphocytes, which has (germline encoded or postthymically altered) a high affinity for viral antigen and a low affinity for H-2 and is normally not stimulated by viral antigen presented in association with H-2 molecules. Stimulation with antiidiotypic antibody might, in such a model, induce T cells with high affinity for the antigen. These unusual clones may be stimulated preferentially by the antiidiotype because of its high affinity for the portion of the receptor (or receptors) which binds antigen. Part of the difference in MHC restriction seen upon challenge with the antiidiotypic antibody may be a result of repertoire differences among mouse strains. Future experiments including analysis of the clones of T cells induced by the antiidiotype as opposed to virus alone may lead to an understanding of whether different cell types are stimulated.

Summary

We used an antiidiotypic antibody directed against a Sendai virus–specific T helper cell clone to stimulate an immune response in vivo. In addition, the antiidiotypic antibody induced T cells that mediated a delayed-type hypersensitivity (DTH) response in several different mouse strains. Induction of the DTH response by the antiidiotypic antibody, in contrast to the DTH responses induced by virus, demonstrated a remarkable lack of H-2 restriction.

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