

ELICITATION OF DELAYED-TYPE HYPERSENSITIVITY
TO PHOSPHORYLCHOLINE BY
MONOCLONAL ANTI-IDIOTYPIC ANTIBODIES
IN AN ALLOGENEIC ENVIRONMENT

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Most T lymphocytes sensitized to an antigen show a specificity which is not directed towards the antigen per se, but rather against the antigen in association with self determinants encoded by the major histocompatibility complex. This phenomenon has been called H-2 restriction in the mouse system (1). Numerous reports have shown that instead of antigen, anti-idiotypic antibodies can also be used for activation of various T cell subsets, e.g., helper cells (2, 3), suppressor cells (4), and T cells involved in delayed-type hypersensitivity (DTH) (5, 6). So far it is not known if this activation occurs in an H-2-restricted manner as well.

To approach this question, we have established a test system in which DTH to phosphorylcholine (PC) can be elicited with monoclonal anti-idiotopes raised against the PC-binding myeloma protein HOPC-8. Transfer of DTH into naïve allogeneic mice by PC-activated T cells and subsequent elicitation with either PC or anti-idiotypic antibodies allowed us to compare the requirements for both types of activation. We demonstrate in this paper that PC-sensitized T cells can be reactivated by anti-idiotypic antibodies in an allogeneic environment.

Materials and Methods

Mice. 8–12-wk-old mice purchased from Olac Ltd., England, and from Zentrale Versuchstieranstalt, Hannover, Federal Republic of Germany (FRG) were used.

Monoclonal Anti-Idiotypic Antibodies. Production and characterization of the monoclonal anti-idiotopes against the PC-binding HOPC-8 myeloma protein have already been described (7). The following antibodies were used (mouse origin of the HOPC-8-sensitized B cells used for fusion and antibody class are given in parentheses): B36-75 (BALB/c, IgG 1), B39-38 (BALB/c, IgG 1), B36-82 (BALB/c, IgM), B24-50 (SJL, IgG 2b), and AB1-2 (A/J, IgG 1). AB1-2 was generously provided by Dr. J. Kearney, Birmingham, AL. The antibodies were purified from culture supernatants or ascites fluid by ammonium sulfate precipitation and affinity chromatography on HOPC-8 conjugated sepharose 4B.

Immunization Procedures and DTH Test. 2 d after cyclophosphamide treatment (100 mg/kg body weight Endoxan [ASTA, Brackwede, FRG] injected subcutaneously) BALB/c mice were sensitized to PC by injecting 4×10^7 haptenated syngeneic spleen cells (SP-PC) subcutaneously into two sites of the hind trunk area. Haptenation was performed either by diazo-coupling of diazonium phenylphosphorylcholine (DPPC) (4) or by chromium-chloride coupling of C-polysaccharide of pneumococcus R36A to the cells. The DPPC was synthesized according to Chesebro and Metzger (8). The C-polysaccharide was obtained according to Liu and Gotschlich (9). For challenge, mice were injected 5 d after sensitization with 10^6 haptenated syngeneic

peritoneal exudate cells (PEC-PC) intradermally in the left ear. Whenever monoclonal antibodies were used instead of antigen for immunization or challenge, they were applied in phosphate-buffered saline at the same sites as described for the haptenated cells. 2–3 h after challenge, DTH was determined by the radioisotopic ear method using ^{125}I -labeled 5-iodo-2'-deoxyuridine as described in detail elsewhere (10). The extent of DTH is expressed as the ratio of the radioactivity in the left to the right ear (L/R ^{125}I -UdR uptake).

Adoptive Transfer of DTH. 5 d after sensitization, regional popliteal, periaortic, and inguinal lymph nodes were removed, and single cell suspensions were prepared. 7×10^7 lymph node cells were injected intravenously into naive recipients, and challenge was performed immediately as described above.

Statistics. Calculations of *P* values according to Student's *t* test were performed. Differences between two groups were not considered significant when *P* > 0.05.

Results

Elicitation of PC-induced DTH by Monoclonal Anti-Idiotypic Antibodies. Cyclophosphamide-treated BALB/c mice were sensitized to PC by injecting 4×10^7 SP-PC subcutaneously. 5 d later, these mice were challenged either with the antigen (10^6 PEC-PC) or with 3 μg of various mixtures of monoclonal anti-idiotypic antibodies. Significant DTH reactions were found in all sensitized animals, whereas in naive mice, no DTH could be elicited (Table I). Normal mouse immunoglobulin G (MIgG), however, could not elicit DTH to PC. There was also no reaction detectable when keyhole limpet hemocyanin (KLH)-sensitized mice were challenged with anti-idiotypic antibodies. Table I (last line) shows also that anti-idiotopes could be used for sensitization.

The PC-induced DTH reaction was dependent on the dose of the anti-idiotypic antibodies used for elicitation (Fig. 1). 3–5 μg of the antibody mixture were optimal in eliciting DTH without raising an unspecific inflammatory response in naive animals. Challenge of PC-sensitized mice with 0.5–5 μg of the various single monoclonal anti-idiotypic antibodies was as inefficient as MIgG. Only challenge with the antibody AB1-2 led to higher DTH values which, however, in some experiments were not reproducibly different to the controls. Mixtures of three or more anti-idiotypic antibodies had to be used to obtain significant DTH responses in PC-sensitized

TABLE I
Elicitation of DTH to PC with Monoclonal Anti-HOPC 8 Antibodies in
BALB/c Mice

Sensitization	Elicitation	L/R ^{125}I -UDR uptake		<i>P</i> values
		Naive	Sensitized mice	
SP-PC*	PEC-PC‡	1.30 ± 0.15	2.40 ± 0.20§	<0.005
SP-PC	3 μg mix 1 ¶	1.20 ± 0.10	2.15 ± 0.10	<0.005
SP-PC	3 μg mix 2 ¶	1.15 ± 0.10	1.95 ± 0.15	<0.01
SP-PC	3 μg MIgG	1.10 ± 0.05	1.05 ± 0.05	>0.05
50 μg KLH	3 μg mix 2 ¶	1.10 ± 0.05	1.05 ± 0.05	>0.05
50 μg KLH	3 μg KLH	1.35 ± 0.20	4.30 ± 0.50	<0.001
20 μg mix 2	PEC-PC	0.95 ± 0.05	1.90 ± 0.20	<0.005

* 4×10^7 syngeneic spleen cells haptenated with C-polysaccharide of R36A.

‡ 10^6 syngeneic, haptenated PEC.

§ Arithmetic mean ± SE; six mice per group.

¶ Mixture of equal amounts of AB1-2, B36-75, B39-38, B24-50, and B36-82 monoclonal anti-idiotypic antibodies.

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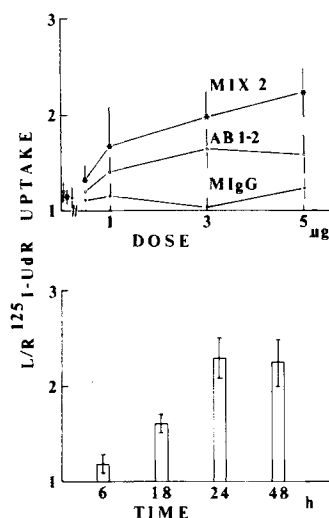


FIG. 1. Elicitation of PC-induced DTH by monoclonal anti-idiotypic antibodies. Top: dose dependence. Mix 2 is a mixture of equal amounts of AB1-2, B24-50, and B39-38 monoclonal antibodies. The unspecific inflammatory response in naïve animals caused by 5 µg of the various antibodies injected in the left ear is given at the start of the ordinate. Bottom: time dependence using 5 µg mix 2. Vertical bars indicate limits of standard error. Five mice per group.

TABLE II
Comparison of the Requirements for T Cell Activation by Either Antigen or Anti-Idiotypic Antibodies in Transfers of DTH

Sensitization of BALB/c by	Elicitation in			
	BALB/c		BALB.K	
	PEC-PC	5 µg mix 2	PEC-PC	5 µg mix 2
SP-PC	1.95 ± 0.30‡	1.80 ± 0.25	1.30 ± 0.15	1.80 ± 0.15
—*	1.20 ± 0.05§	1.05 ± 0.05§	1.35 ± 0.15	1.15 ± 0.10§
50 µg mix 2	1.70 ± 0.15	ND¶	1.25 ± 0.10	ND
—*	1.20 ± 0.10§	ND	1.25 ± 0.05	ND

Conditions for sensitization and elicitation as in Table I. Naïve BALB/c or BALB.K recipients received 70×10^6 lymph node cells from sensitized BALB/c mice and were immediately challenged with either PC-modified BALB/c PEC or with a mixture of equal amounts of AB1-2, B24-50, and B39-38 monoclonal anti-idiotypic antibodies (mix 2).

* As controls for the elicitation reaction, naïve mice were challenged in the same way as the recipients of the sensitized lymph node cells.

‡ Arithmetic mean ± SE; five mice per group.

§ P values for difference between naïve and corresponding sensitized group range from <0.005 to <0.05.

|| P value for difference between naïve and corresponding sensitized group >0.05.

¶ Not determined.

mice. The time dependence of the elicitation clearly showed a delayed reaction, with a peak at 24–48 h (Fig. 1).

Transfer of PC-induced DTH in Allogeneic Mice and Elicitation by Monoclonal Anti-Idiotypic Antibodies. 7×10^7 lymph node cells of PC-sensitized BALB/c mice were transferred into either syngeneic BALB/c (H-2^d) or allogeneic BALB.K (H-2^k) mice 5 d after sensitization. Mice were challenged either with antigen (10^6 PEC-PC from BALB/c)

or 5 μg of a mixture of equal amounts of AB1-2, B24-50, and B39-38 anti-idiotypic antibodies (Table II). In BALB/c mice, DTH could be elicited with antigen as well as with anti-idiotypic antibodies. In allogeneic BALB.K mice, however, DTH could only be seen after challenge with anti-idiotypic antibodies. This finding could be reproduced in three independent experiments. On the other hand, if 50 μg of a mixture of three monoclonal anti-idiotypic antibodies were used to sensitize BALB/c mice, and the lymph node cells of these animals were transferred into either BALB/c or BALB.K mice, subsequent challenge with PC-modified BALB/c PEC led to a DTH reaction only in the syngeneic combination, and not in an allogeneic environment.

Discussion

Anti-idiotypic antibodies can be used to elicit DTH responses induced by the respective antigen. This was already shown with a conventional anti-idiotypic serum against anti-poly-(L-Tyr, Glu)-poly-D, L-Ala--poly-L-Lys (T, G)--A-L antibodies (11). We have used monoclonal antibodies against the PC-binding myeloma protein HOPC-8 to elicit DTH to PC. Application of only one monoclonal antibody to PC primed mice did not lead to a detectable reaction. The use of three or more anti-idiotypic antibodies, however, resulted in a specific delayed reaction that was dose dependent and transferrable by T cells (data not shown). These data may suggest that either several monoclonal anti-idiotopes are necessary to activate one particular T cell clone or, more likely, that the idiotopes are clonally distributed on T cells. Hence, it could be argued that the single monoclonal antibodies we used reactivated too few PC-sensitized T cell clones, whereas the sum of T cell clones reactivated by three or more monoclonal antibodies was sufficient to result in a positive DTH response. This interpretation would be supported by the finding that most PC-specific B cell hybridoma antibodies express only one or the other but rarely all of the idiotopes defined by our monoclonal anti-idiotopes (R. Wallich, G. J. Hämmerling, and P. J. Gearhart, manuscript in preparation).

Using transfer studies, we found that the requirements for the reactivation of DTH T cells by either antigen or anti-idiotypic antibodies are different. We could confirm the finding by Thomas et al. (6), that whenever antigen is used for the elicitation of DTH H-2 restriction is seen, regardless of whether the DTH T cells were activated by antigen or anti-idiotypic antibodies. Because the PC-modified BALB/c PEC could not reactivate the sensitized and transferred BALB/c T cells in BALB.K mice, one could assume that either haptenation disturbed the ability of the PEC to present antigen, or among the 10^6 PC-PEC, too few antigen-presenting cells were injected into the ear. On the other hand, the anti-idiotypic antibodies could reactivate antigen-sensitized T cells in an H-2 incompatible host. These findings may be explained if one assumes that successful activation of T cells depends on the affinity of the interaction between the T cell recognition structure and ligand, e.g., antigen or anti-idiotope. If antigen is used, then the affinity of the T cell receptor is only high enough for antigen in association with syngeneic MHC determinants. In contrast, if anti-idiotope is used, then the affinity of this reagent is the decisive factor. However, it should be kept in mind that our results do not prove that anti-idiotypic antibodies react directly with T cells, nor do they answer the question of whether the recognition structure of T cells is controlled by immunoglobulin heavy chain variable region genes.

Summary

Delayed-type hypersensitivity (DTH) to phosphorylcholine (PC) could be elicited by mixtures of monoclonal anti-idiotypic antibodies. Using this system in DTH transfers, the question was asked of whether anti-idiotypic antibodies could elicit antigen induced DTH in H-2-incompatible mice. Transfer of PC-activated BALB/c lymph node cells into BALB.K mice and subsequent elicitation with anti-idiotypic antibodies resulted in a positive DTH response. In contrast, elicitation with the antigen PC showed the expected H-2 restriction.

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