Mechanisms of Genetic Control of Immune Responses

II. Nonresponsiveness in BALB/c GT-Specific Cell-Mediated Immune Responses Does Not Correlate with the Absence of Functional T Cells or the Induction ofSuppressor T Cells

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Abstract. The mechanisms underlying Ir gene control of CMI were addressed by examining the DTH and Tprlf responses specific for the synthetic polymers GT, GAT, and GA. We show that BALB/c mice (GAT/GA responders, GT nonresponders) primed with GT fail to develop DTH and Tprlf responses specific for GT, GAT, or GA. GAT immunization resulted in DTH responses that could be elicited not only with GAT and GA but also with GT, demonstrating that GT-specific T cell responses are present in nonresponder mice. GT-specific DTH was transferred with Thy-1+ Lyt-1+2-, H-2I- restricted, nylon wool nonadherent cells. GA-primed BALB/c mice developed GAT- and GA-, but not GT-specific DTH responses, indicating that GA and GT do not cross-react at the T-cell level. The ability of GAT [but not a mixture of GA plus GT, or GT electrostatically complexed to the immunogenic carrier MBSA (GT-MBSA)] to induce GT-specific DTH suggested a requirement for covalent linkage of stimulatory 'GA' and nonstimulatory 'GT' determinants present on the GAT molecule. Similarly, GT-specific in vitro Tprlf responses could be demonstrated in GAT-primed mice exhibiting significant levels of GT-specific DTH but not in GT- or GT-MBSA-primed mice. Tolerization experiments also suggested that GT-specific Th were involved in the development of GT-specific DTH in GAT-primed mice. The GT nonresponsiveness of BALB/c mice for DTH and Tprlf responses could not be reversed by treatments designed to abrogate Th activity (priming with GT-MBSA and CY injection), nor could GT-primed cells be shown to inhibit the development or elicitation of GT-specific CMI in GAT-primed mice during the afferent and/or efferent stages of DTH. Our results suggest that GT nonresponsiveness does not result from the absence of GT-specific T cells or preferential induction of Ts. The results are discussed in the context of hole-in-the-repertoire and antigen presentation (determinant selection) models of Ir gene control.

Introduction

Mechanisms of Ir gene control of immune responses can be viewed in the context of two main theories (Schwartz 1982). The determinant selection theory proposes a specific interaction between nominal antigen and H-2 determinants on the surface of APC in which nonresponsiveness results from the inability of antigen/H-2 to associate in an immunogenic form. Hole-in-the-repertoire theories propose that nonresponsiveness results from the absence of functional T-cell clones capable of recognizing a given antigen/H-2 complex due to functional or physical deletion during self-tolerance induction, lack of the appropriate T-cell receptor genes, or masking by suppression.

Studies of immune responses to synthetic polypeptide antigens of limited heterogeneity such as GAT and GT have provided much information on Ir gene control. Humoral and cellular immune responses to GAT are controlled by products of the A subregion; mice of the H-2^a, H-2^b, H-2^k, and H-2^e haplotypes develop specific humoral and cellular responses following GAT priming (Dunham et al. 1973, Kapp et al. 1973a, Miller et al. 1977a, Schwartz and Paul 1976). Mice of the H-2^a, H-2^e, and H-2^k haplotypes fail to develop these responses. This is partly due to the induction of Ts and their soluble products (Kapp et al.
1973b, Germain and Benacerraf 1980). All inbred strains of mice, with the exception of the bm12 mutant, are GT nonresponders, as determined by specific plaque-forming cell (PFC) responses (Lei et al. 1982). Although Ts activity has been clearly demonstrated in some GT nonresponder strains (H-2d, H-2k, H-2s), the existence of GT nonresponders in which Ts cannot be demonstrated (H-2b, H-2k, H-2s) suggested that the Ir defect(s) is not due solely to Ts activation (Debre et al. 1975a, b, 1976a). Nonresponder mice treated with CY (to eliminate Ts precursors) prior to the injection of GT or GAT develop weak specific PFC responses, suggesting that nonresponders possess Th capable of recognizing these antigens (Debre et al. 1976b, Miller et al. 1984). In contrast, CY treatment of GT nonresponders does not lead to DTH or Tprlf responses following GT injection (Miller et al. 1984). Thus, the existence of GAT/GT-specific Th, Tprlf, and TDP in nonresponders remains unclear.

The present study addresses the nature of the Ir gene defect in GT-specific CMI. GT-nonresponder BALB/c mice primed with GT or GT-MBSA fail to develop GT-specific DTH or Tprlf responses. Treatments designed to abrogate or circumvent Ts activity failed to reveal specific DTH responses in GT-primed BALB/c nonresponders. In contrast, BALB/c mice primed with GAT but not with the related copolymer GA, or simultaneously with GA plus GT, developed significant GT-specific DTH and Tprlf responses. Collectively, these results indicate that GT-specific T cells are present in the T-cell repertoire of nonresponder BALB/c mice and that the failure to develop GT-specific DTH following GT priming cannot be ascribed to preferential Ts induction.

Materials and Methods

**Mice.** Female BALB/c mice were purchased from Cumberland View Farms, Clinton, Tennessee. C57Bl/6, B10.D2, B10.A, and B10.LG mice were kindly provided by Dr. Roger Melvold (Northwestern University, Chicago). BALB.B mice were bred and housed in the Northwestern University animal care facility. All mice were maintained on standard laboratory chow and water ad libitum. Mice were age-matched (8-12 weeks) within experiments.

**Antigens.** Antigens were purchased from the following sources: GAT (lot 31F-5040), GT (lot 51F-5054), and BSA, (fraction V; Sigma Chemical Company, St. Louis, Missouri); GA (lot GA3; Miles Laboratories, Elkhard, Indiana); and PPD (Connaught Laboratories, Elkhart, Indiana) two days prior to immunization. All inbred strains of BALB/c mice were kindly provided by Dr. Roger Melvold (Northwestern University, Chicago). BALB.B mice were bred and housed in the Northwestern University animal care facility. All mice were maintained on standard laboratory chow and water ad libitum. Mice were age-matched (8-12 weeks) within experiments.

**Immunizations and elicitation of DTH.** To induce DTH and Tprlf responses, mice were injected i.p. with 20 mg/kg CY (Meade Johnson & Co., Evansville, Indiana) two days prior to immunization. Six to seven days after priming, DTH responses were elicited by injecting 1-10 µg of antigen (diluted in 10 µl of saline) into the dorsal surface of the ear using a 100 µl Hamilton syringe fitted with a 30 gauge needle. Twenty-four hours after each challenge, the increase in ear thickness over prechallenge measurements was determined using a Mitutoyo model 7326 engineer's micrometer (Schlesinger's Tools, Brooklyn, New York) and was expressed in units of 10⁻² inches.

**Transfer of DTH.** T_DTH donor mice were injected i.v. with 2.5 x 10⁶ heat-killed Bordetella parapertussis organisms (Michigan Department of Health) immediately prior to sensitization by s.c. injection of a total of 40 µg GAT/CFA or GT/CFA (20 µg at the tail base and 5 µg in each footpad). Seven days post-sensitization, draining LNs (inguinal, paraortic, brachial, axillary, and popliteal) were removed, single cell suspensions were prepared, and 4-5 x 10⁵ viable cells were transferred i.v. to groups of 3-4 normal recipients. Recipient mice were challenged within 1 h of cell transfer and the degree of DTH was measured 24 h later. Nylon wool column fractionation was performed according to the method of Julius and co-workers (1973).

**Preparation of antigen-coupled spleen cells.** Erythrocyte-free (Tris-NH₄Cl-treated) spleen cells were coupled with protein antigens as previously described (Miller et al. 1979). Washed spleen cells (4 x 10⁸) were resuspended in 1.5 ml of a 2 mg/ml solution in saline (pH 7.0) of GAT, GA, GT, or BSA. A quarter of a milliliter of water-soluble carbodiimide HCl (100 mg/ml in saline) (catalog no. 341006, Calbiochem-Behring Corp., La Jolla, California) was added, and the suspension was incubated for 1 h on ice. Antigen-coupled cells were washed three times with BSS and kept on ice until used. Trace labeling with ¹²⁵I-antigen showed that approximately 3-5 µg of antigen was coupled to 10⁶ spleen cells.

**Induction of tolerance.** Tolerance was induced by i.v. injection of 5 x 10⁶ antigen-coupled spleen cells (Miller et al. 1979, Jenkins et al. 1984). Seven days later, tolerant and control (receiving no cells i.v.) mice were immunized with 20 µg GAT/CFA at the tail base. Six days postimmunization, mice were challenged with 10⁻³ antigen in 1 h later. nylon wool column fractionation was performed according to the method of Julius and co-workers (1973).

**In vitro Tprlf.** Following the determination of DTH, 4 x 10³ draining LN cells (inguinal and paraaortic) were cultured in 96-well flat-bottom microtiter plates (Falcon 3072) in a volume of 0.2 ml of modified Click's medium containing 5 x 10⁻³ M 2-mercaptoethanol, 0.01 M NaHCO₃, and 0.5% fresh, syngeneic mouse serum (Corradin et al. 1977). Cultures were stimulated with the indicated doses of antigen and incubated in a humidified atmosphere of 5% CO₂, 95% air. After 96 h, cultures were pulsed with 1.0 µCi of ³H-thymidine (³H-Tdr, specific activity 6.7 Ci/mM; Research Products International Corp., Mount Prospect, Illinois). Cultures were harvested 24 h later with a semiautomated sample harvester, and measurements of trichloroacetic acid-precipitable material were determined in a liquid scintillation counter. Cultures were performed in triplicate. Results are expressed as (mean cpm of antigen-containing cultures) - (mean cpm of antigen-free controls) as previously described (Miller et al. 1979).

**Antiserum treatments.** Monoclonal immunoglobulin M (IgM) anti-Thy-1.2 and Lyt-1.2 ascitic fluids were purchased from New England Nuclear Corp., Boston, Massachusetts, and monoclonal IgM anti-Lyt-2.2 ascitic fluid (clone ADH4.15) was kindly supplied by Dr. P. Marrack, The National Jewish Hospital and Research Center, Denver, Colorado. Lymphoid cells were treated at a concentration of 10⁶/ml of antibody diluted to 1:200 for 45-60 min at 4°C. The cells were washed once at 4°C in BSS, resuspended in 1.0 ml of a 1:6