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17. The linked dimers **1** and **2** were prepared as follows. We prepared gA from the commercially available mixture (gA′) by flash chromatography on silica gel, using chloroform:methanol:water:acetic acid (~400:30:4:1) [for further details see (*16*)]. gA was then deformylated (*1*) with anhydrous hydrogen chloride in methanol (generated from acetyl chloride and methanol). Desformyl gA was purified on AG-MP-50 and Sephadex LH-20. The coupling was accomplished according to the method of Weiss and Koeppe (*15*). DPPA was added to a solution of the bis-acid and desformyl gA in *N*,*N*-dimethylformamide (DMF) at −20°C, and then triethylamine was added. The resulting solution was kept at 0°C for 24 to 48 hours, then warmed to room temperature and quenched with methanol. The DMF was then removed by vacuum evaporation. Purification of the crude product by Sephadex LH-20, ion-exchange chromatography with AG-MP 50, silica gel chromatography, and ion-exchange chromatography with AG-501 yielded the desired dimers. A sample of these products was further purified by reversed-phase HPLC on a Beckman C-18 column (10 by 250 mm) with methanol:water (95:5) at 3 ml/min to provide a highly purified sample for conductance studies.
18. The ^{1}H NMR spectra of both dimers are nearly identical to that of gA (*7*) except for the appearance of two new singlets, associated with the linker, with the expected integrations. Spectra were recorded on a Bruker 500-MHz ^{1}H NMR in DMSO-d_6 with δ values reported relative to DMSO-d_5(H) (DMSO, dimethyl sulfoxide). (*S*,*S*)-linked dimer **1**: δ 5.15 (s, 2H, OCH_2O), 4.65 (s, 2H, CH). (*R*,*R*)-linked dimer **2**: δ 5.1 (s, 2H, OCH_2O), 4.55 (s, 2H, CH). In each case the signal for the hydroxyl proton for the ethanolamine is still present and integrates correctly for two protons, indicating that these dimers are linked amino terminus to amino terminus. The R_f values in chloroform:methanol:water:acetic acid (100:30:4:1) are as follows: gA (0.71), desformyl gA (0.37), (*S*,*S*)-linked dimer **1** (0.20), and (*R*,*R*)-linked dimer **2** (0.66). S. Weinstein, J. T. Durkin, W. R. Veatch, and E. R. Blout [*Biochemistry* **24**, 5249 (1984)] have used the ratio of the absorbance at 320 nm to that at 290 nm as an indication of the absence of degradation of the tryptophans in gA. For the linked dimers, the corresponding ratios [(*S*,*S*)-linked dimer **1** (0.033) and (*R*,*R*)-linked dimer **2** (0.061)] indicate that little if any appreciable degradation has occurred. We have observed that this degradation can also be estimated by analysis of the aromatic region of the ^{1}H NMR spectra, which shows new signals for the degradation product.
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Class II MHC Molecules Are Specific Receptors for Staphylococcus Enterotoxin A

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T cell proliferation in response to stimulation with *Staphylococcus* enterotoxin A (SEA) requires accessory cells that express class II major histocompatibility complex (MHC) molecules. Murine fibroblasts transfected with genes encoding the α and β subunits of HLA-DR, DQ, or DP were used to show that the proliferative response of purified human T cells to SEA is dependent on class II molecules but is not restricted by the haplotype of the responder. Binding of fluoresceinated SEA to class II transfectants and precipitation of class II heterodimers with SEA-Sepharose show that the proliferative response is a result of SEA binding to class II molecules. The binding is specific for class II molecules and is independent of class II allotype or isotype. The ability of SEA to bind class II molecules may be a general characteristic of this class of antigens, now called "superantigens."

STAPHYLOCOCCAL ENTEROTOXINS ARE a family of molecules, encoded by bacteriophages, that cause staphylococcal food poisoning (*1*). These proteins are also powerful T cell mitogens; like other mitogens they cause T cell activation and proliferation in the presence of accessory cells (*2*). However, unlike most mitogens, SEA also requires cells with MHC class II molecules on their surface for presentation to T cells (*3, 4*). Because protein antigens, but not mitogens, are dependent on interactions with MHC class II molecules for presentation to and activation of $CD4^+$ T cells, we investigated the role of class II molecules in the T cell reponse to SEA.

Because class II antigen expression is characteristic of most conventional antigen-presenting cells, we used mouse fibroblasts transfected with both HLA class II α and β chains to assess the role of these molecules independent of the accessory cell signals supplied by the fibroblasts. Purified T cells from one individual were tested with a panel of transfected fibroblasts that includes members of the three isotypes of HLA class II antigens (DR, DQ, and DP) (Fig. 1). Each of the transfected fibroblasts supported proliferative responses to SEA, regardless of the allotype or class II isotype of the transfected gene. Untransfected cells and HLA class I–transfected fibroblasts did not support proliferative responses at any SEA concentration. All of the fibroblast lines similarly supported a phytohemagglutinin response, whether or not class II molecules were present.

These data, along with reports that SEA does not require cellular processing to stimulate T cells (*3*), were indicative of the possibility that SEA binds directly to class II molecules to form a complex that interacts with T cell receptors in a largely MHC-unrestricted fashion. We therefore used flow cytometry to assess the binding of fluoresceinated SEA (Fig. 2). SEA could bind to the human B cell line Raji but not to RJ2.2.5, a class II–negative mutant of this line that expresses class I molecules but does not express any of the three class II isotypes (*5*). Similarly, SEA could bind to HLA-DR1–transfected fibroblasts but not to the untransfected parental cells. Specific binding to resting T cells was not observed. The capacity to bind class II antigens was also tested by immunoprecipitation with SEA from detergent lysates of cells surface-labeled with ^{125}I. SEA bound two proteins from the B cell line Raji that migrated with DR α and β polypeptides that could be precipitated with L243, a monoclonal antibody to the class II DR isotype (Fig. 3) (*6*). Neither SEA nor L243 precipitated these proteins from the class II–negative mutant RJ2.2.5. Similarly, SEA bound the class II heterodimer from mouse fibroblast lines transfected with either of two different DR alleles, or with DQ or DP (*7*). We investigated the possibility that SEA also bound a ligand on the responding cells—for example, the T cell receptor. However, SEA did not bind any iodinated cell surface proteins from purified T cell lysates (Fig. 3), indicating that native SEA does not bind the T cell receptor or other cell surface molecules with a high-affinity interaction similar to that involved in binding class II molecules. This does not rule out the possibility that SEA may bind directly to T cell receptors with a substantially lower affinity or to a subset of

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T cell receptors as has recently been proposed (*4*), in which case the sensitivity of the assay may be insufficient. Because SEA is also a murine T cell mitogen (*2*), we tested it with mouse spleen cells and found that SEA also binds mouse class II molecules, precipitating a band that comigrated with the immunoprecipitated I-E β chain (Fig. 3); the absence of the I-E α chain is due to its characteristic failure to iodinate (*8, 9*). These data demonstrate that the ability of SEA to bind class II molecules lacks both species specificity and specificity for a particular HLA allele or class II isotype.

The highly polymorphic nature of MHC class II molecules makes them improbable candidates for use as a receptor by a bacteriophage product. However, SEA appears to use a binding site on the class II antigen that shows little or no variation among allotypes, isotypes, or species as diverse as mouse and human. SEA may bind to a relatively monomorphic region of class II molecules such as that utilized by the CD4 molecule (*10*), or may bind class II molecules near their putative peptide-binding cleft in a manner that allows co-recognition of SEA and the MHC element. It is possible that one domain of SEA binds to the class II molecule and the other to the T cell receptor. In any case, our preliminary data suggest that SEA binds to a conformational determinant formed by both the class II α and β chains and not either chain independently.

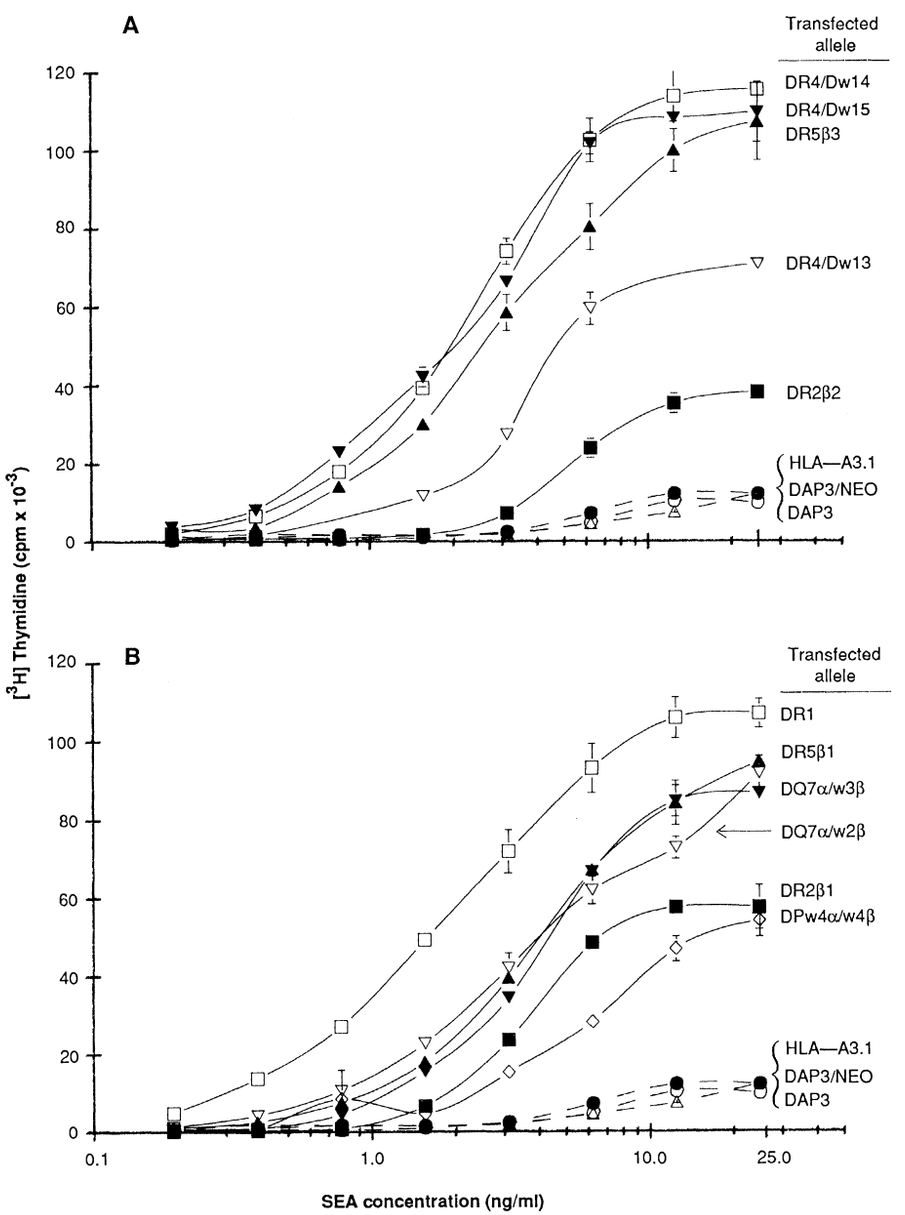

Fig. 1. Mouse fibroblasts transfected with HLA class II, but not class I, molecules support human T cell proliferative responses to SEA (*16*). (**A** and **B**) Data from a single experiment. The HLA haplotype of the responder was DR2(DQw1); DR4(DQw3:DRw53); A1,A11; and B8(w6),B37(w4). The data are representative of four experiments with cells from different individuals (*17*), expressing distinct HLA haplotypes. Values are means ± SEM.

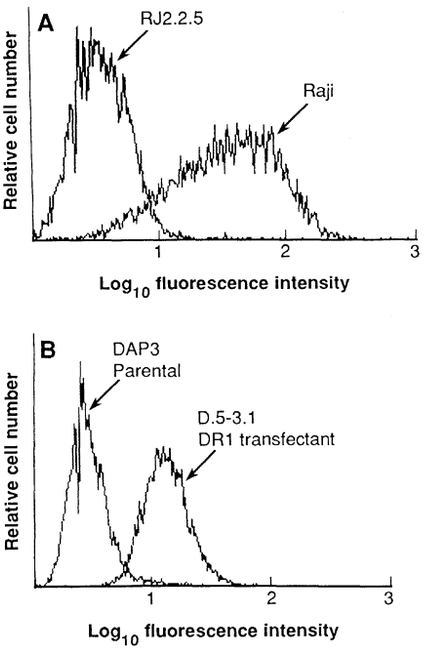

Fig. 2. Fluorescein-conjugated SEA stains two class II–positive cell lines but not their respective class II–negative controls (*18*). (**A**) Fluorescence histograms of cell lines Raji and RJ2.2.5 (class II–negative mutant) stained with SEA-FITC. (**B**) Fluorescence histograms of the DR1-transfected fibroblast line (D.5-3.1) and its untransfected parental cell line DAP3.

A striking characteristic of the T cell response to SEA is that it appears unrestricted to the haplotype of the responding individual (*11*), a hallmark of T cell receptor–mediated antigen recognition. The involvement of MHC molecules and blocking of SEA responses by antibodies (OKT3) directed against the CD3 component of the T cell receptor (*12*) suggest that T cells recognize the SEA–class II complex via their antigen receptor rather than an alternative activation pathway. This supports conclusions of White *et al.* (*13*) and Janeway *et al.* (*4*) on mouse T cell responses to staphylococcal enterotoxins. We used purified resting T cells as responders instead of T cell clones to avoid the confounding factor of autologous class II antigens expressed by cloned human T cells. However, the data in Fig. 1 demonstrate that T cells respond to SEA in the context of many distinct class II types and alleles. We do not know whether the T cells that are responding to SEA in the context of different class II alleles are separate or overlapping populations. One explanation for heterogeneity in the level of responses supported by different transfectants is that different populations of T cells respond to different SEA/MHC combina-

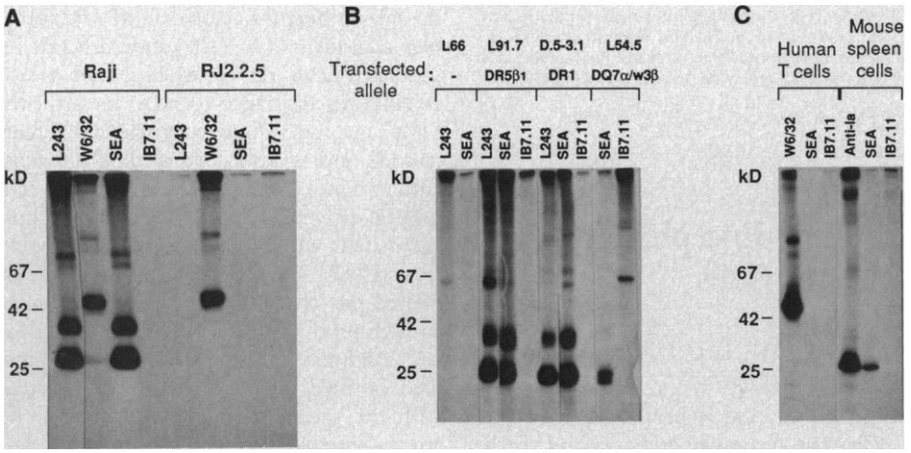

Fig. 3. SEA precipitates class II heterodimers from detergent lysates of B cells and HLA-DR- and DQ-transfected fibroblasts (*19*). SDS-PAGE analysis of proteins precipitated from detergent lysates of the indicated cell lines with monoclonal antibodies or SEA conjugated to Sepharose. (**A**) Raji and RJ2.2.5 (class II–negative mutant). (**B**) Fibroblast lines L66 (Neo only) , L91.7(DR5β1), D.5-3.1(DR1), and L54.5(DQ7α;DQw3β). (**C**) Purified T cells and BALB/c spleen cells. Antibodies used were L243 (anti–HLA-DR), W6/32 (anti–HLA-class I), and 1B7.11 (anti-trinitrophenyl). The α chain of the DQ transfectant in (B) is apparent in autoradiographs exposed for longer times.

tions. Alternatively, SEA may bind more effectively to some alleles than to others. Variable levels of class II expression do not appear to be a factor. For example, the DR2 β_2 transfectant consistently supports a much lower SEA response than the DR1 transfectant although these lines express similar levels of class II molecules. We have not yet found a class II allele that does not support an SEA response, nor have we found an allele unable to bind SEA.

White *et al.* (*13*) showed that *Staphylococcus* enterotoxin B (SEB) is recognized by the subsets of murine T cells that utilize the $V_\beta 3$ and $V_\beta 8$ T cell receptor genes, whereas SEA was recognized by T cells bearing $V_\beta 3$ and $V_\beta 11$. These authors termed the enterotoxins "superantigens" because of the ability of the enterotoxins to interact with large numbers of T cell receptors on the basis only of Vβ usage and because they have a less restricted pattern of recognition by T cells. We suggest that another essential characteristic of these superantigens may be their capacity to bind class II molecules with high affinity in a way that overrides MHC-restricted recognition.

These data provide new insights into mechanisms whereby immune responses to SEA and similar bacterial exotoxins differ from those to most protein antigens. The ability of SEA to bind class II molecules and activate large numbers of T cells with overlapping specificities raises the possibility that exposure to such bacterial products in vivo may underlie diseases, including several autoimmune disorders in which T cells that share common receptor determinants are activated (*14*).

The hypothesis that MHC class I and II molecules are receptors for bacterial products has long been postulated, for example, in the pathogenesis of ankylosing spondylitis (*15*). Nevertheless, direct demonstration that MHC molecules serve as specific receptors for bacterial products has been lacking. MHC gene products play a central role in determining the immune response to any antigen. Our report that SEA binds directly to class II molecules, with consequential T cell activation, is an example of how MHC molecules may interact with the environment to determine the outcome of an immune response.

Note added in proof: Similar data concerning the binding of SEA to HLA-DR have recently been reported (*20*).

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18. SEA (250 μg) was suspended at a concentration of 1 mg/ml in 0.05*M* bicarbonate buffer and mixed with 125 μg of fluorescein isothiocyanate (FITC) and celite (Sigma). The mixture was rocked for 90 min at room temperature in the dark. Excess FITC-celite was removed by centrifugation and the SEA-FITC mixture was dialyzed against phosphate-buffered saline (PBS) to remove free FITC. The FITC-SEA mixture was titrated against class II–transfected fibroblasts and stained optimally at a 1:40 dilution per 0.5×10^6 cells.
19. Antibodies were conjugated to Sepharose at concentrations of 4 mg/ml (L243, 1B7.11), 2 mg/ml (W6/32), and 1 mg/ml (SEA) of CNBr-activated Sepharose. I-E was immunoprecipitated with the A.TH-anti-A.TL antiserum (NIAID Serum Bank) plus staphylococcal protein A–agarose (Pierce Chemicals). For surface labeling with ^{125}I, 20×10^6 B cells and fibroblasts and 30×10^6 spleen cells and T cells were washed twice in PBS and labeled with 2 mCi of ^{125}I by the lactoperoxidase method [J. J. Marchalonis *et al.*, *Biochem. J.* **124**, 921 (1971)]. The cells were lysed for 45 min in 0.5% NP40/TBS with protease inhibitors. Lysates were dialyzed against tris-buffered saline precipitated with 25 μl of each of the indicated Sepharose preparations and rocked for 8 hours at 4°C. Afterward, the Sepharose was washed, then suspended in 50 μl of Laemmli solubilizing buffer [U. K. Laemmli, *Nature* **227**, 680 (1970)] under nonreducing conditions, and boiled for 5 min; supernatants were loaded onto a 10% polyacrylamide gel and subjected to electrophoresis

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Control of Experimental Autoimmune Encephalomyelitis by T Cells Responding to Activated T Cells

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T cell vaccination against experimental autoimmune disease is herein shown to be mediated in part by anti-ergotypic T cells, T cells that recognize and respond to the state of activation of other T cells. The anti-ergotypic response thus combines with the previously shown anti-idiotypic T cell response to regulate autoimmunity.

AUTOIMMUNE DISEASES SUCH AS EXperimental autoimmune encephalomyelitis (EAE) are caused by T cells expressing two attributes: receptors for the specific self antigen, which identify the target tissue, and a state of functional activation, which is a prerequisite for attack. The self-antigen in EAE is the basic protein (BP) of central nervous system myelin (*1*). EAE can be suppressed by anti-idiotypic T cells that recognize the specific anti-BP receptors (the idiotypes) of the autoimmune T cells (*2, 3*). We now report the control of EAE by T cells that recognize not the idiotype of the autoimmune T cells, but a marker of their state of activation. These T cells, which we term anti-ergotypic T cells [εργον (ergon) = work, action] were induced by vaccinating Lewis rats with activated cells of syngeneic T cell clones lacking receptors for BP. In contrast to anti-idiotypic T cells, defined by a response to the specific idiotype borne by the anti-BP T cells, the anti-ergotypic T cells responded to activated T cells in general, without regard for their idiotypic specificities. Anti-ergotypic T cells could also be detected after immunization with antigen in vivo, suggesting that they may function physiologically. Administration of anti-ergotypic T cells to syngeneic rats protected the rats against EAE induced either by adoptive transfer of activated anti-BP clones or by active immunization to BP.

We undertook this investigation as a result of our study of the use of T cell vaccination to induce resistance to EAE and other experimental autoimmune diseases in rats and mice (*4*). We found that autoimmune diseases such as EAE (*5*), thyroiditis (*6*), or adjuvant arthritis (*7*) could be prevented or treated by administering virulent autoimmune T cells specific for the target antigens under circumstances in which the T cells were rendered avirulent. T cell vaccination was also found to be effective against collagen II arthritis (*8*) and against experimental autoimmune neuritis (*9*). Anti-idiotypic immunity to the autoimmune T cell receptor was associated with the resistance induced by T cell vaccination (*2*). Indeed, a clone of anti-clonotypic T cells was shown to mediate resistance to EAE (*3*).

However, several observations suggested that immunity to T cell receptors might not be the only protective element induced by T cell vaccination. First, T cells were efficient vaccines only after they had been activated by incubation with specific antigen or a T cell mitogen before injection (*4, 10*). For example, as few as 10^4 activated anti-BP T cells (*2*) were more effective than 5×10^7 idiotype-positive anti-BP T cells that had not been activated. Therefore, some change in T cells associated with activation is important in the introduction of T cell vaccination; the presence of the idiotype alone is insufficient.

Second, in addition to the high degree of disease-specific protection mediated by T cell vaccination, we observed a mild degree of nonspecific protection. For example, Lewis rats are most effectively vaccinated against EAE by use of activated Z1a T cell clones. Z1a recognizes the immunologically dominant epitope present in the 68– to 88–amino acid sequence of BP (*11*). This clone can either produce EAE or vaccinate rats against EAE, depending on treatment of the cells and the number of cells administered (*10, 12*). In contrast to Z1a, clone A2b (*13*) recognizes a nine–amino acid sequence in the 65-kD heat shock protein of *Mycobacterium tuberculosis* (*14*). A2b causes arthritis in irradiated Lewis rats or, when suitably treated, induces resistance to adjuvant arthritis (*15*). Thus, each clone recognizes a different epitope and is associated with a different autoimmune disease. Nevertheless, when groups of five rats each were or were not vaccinated with 2×10^7 glutaraldehyde-treated (*15*) Z1a or A2b clones, the A2b-treated rats clearly showed some resistance to EAE produced by 2×10^6 activated Z1a cells. All five of the unvaccinated control rats died of EAE, whereas the rats vaccinated with the specific anti-BP Z1a clone were markedly resistant and developed barely detectable clinical disease. Vaccination with clone A2b did not prevent severe EAE, but it did prevent lethal EAE; none of the A2b-treated rats died. Thus, nonspecific vaccination with activated A2b induced significant resistance, albeit a resistance that was not as effective as that obtained by vaccination with clone Z1a.

Our next experiments were undertaken to identify the mediators of resistance to EAE induced by vaccination with T cells that do not recognize the BP antigen. We assayed the delayed type hypersensitivity (DTH) reaction of rats to T cell clones after vaccination (Table 1). DTH reactions are a convenient in vivo measure of T cell reactivity to antigens or idiotypes (*15*). Lewis rats were vaccinated with clone D9, a subclone of line Z1a, to induce optimal resistance to EAE, and the responses of the rats were tested by eliciting DTH reactions to either nonacti-

Table 1. DTH reaction of D9-vaccinated rats to activated and nonactivated T cell clones D9 and A2b. Groups of five Lewis rats each were vaccinated with three weekly injections of 2×10^7 antigen-activated, glutaraldehyde-treated D9 cells (a subclone of Z1a) as described (*15*). DTH was elicited by injecting 3×10^5 irradiated (2500 R) cells in 50 μl of phosphate-buffered saline into the pinna. Activated cells were taken after 72 hours of antigen stimulation. Activation was assessed microscopically and confirmed by [^{3}H]thymidine incorporation. Nonactivated cells had been kept in IL-2–containing medium without antigen for at least 5 days. Ear swelling was determined after 48 hours by use of an isotonic caliper. Values are means ± SE.

State	Clone	DTH as measured by change in ear size (10^{-2} mm)	
		Naïve rats	D9-vaccinated rats
Nonactivated	D9	5.2 ± 2.1	12.3* ± 2.1
	A2b	5.4 ± 1.8	5.5 ± 2.0
Activated	D9	14.1 ± 3.0	33.1 ± 2.9
	A2b	14.8 ± 3.2	26.2 ± 3.2

*$P < 0.05$ compared to response to A2b.

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