Protective Cytotoxic T Lymphocyte Responses Induced by DNA Immunization against Immunodominant and Subdominant Epitopes of Listeria monocytogenes Are Noncompetitive

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Taking advantage of the fact that plasmid DNA encoding a single cytotoxic T lymphocyte (CTL) epitope can induce CTLs, we examined the influence of T-cell responses to dominant epitopes on those to a subdominant epitope derived from Listeria monocytogenes. Our data suggest that interaction between T cells against dominant and subdominant epitopes does not operate in the generation of the hierarchy. Furthermore, we found that a single dominant epitope is sufficient for the induction of protective immunity.

Listeria monocytogenes is a gram-positive bacterium that causes life-threatening infections during pregnancy and in immunocompromised individuals (14). L. monocytogenes enters macrophages and hepatocytes and accesses the cytoplasm by secreting listeriolysin O (LLO), resulting in the induction of a vigorous MHC class I-restricted CTL response that enables the rapid clearance of live bacteria (3, 16). Various factors have been implicated to determine the position of an epitope in the immunodominant hierarchy: (i) antigen processing efficiency (10, 11, 20), (ii) transporter associated with antigen processing (TAP)-dependent peptide transport (15), (iii) the affinity of the peptide for the major histocompatibility complex (MHC) molecule (6, 24), (iv) the rate of dissociation of the MHC molecule (26, 28), (v) the transport of MHC-peptide complexes to the cell surface (17), and (vi) the response to the T-cell repertoire (5, 7, 8).

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Effector CD8+ T-cell responses against infection are restricted to a few epitopes compared to the total number of epitopes potentially available (1, 2, 12, 23). These epitopes are termed immunodominant, and cytotoxic T lymphocyte (CTL) responses to subdominant epitopes can be demonstrated in vivo by the removal of dominant epitopes or the separation of dominant and subdominant epitopes so that they are presented on different cells (9, 18, 21). Various factors have been implicated to determine the position of an epitope in the immunodominant hierarchy: (i) antigen processing efficiency (10, 11, 20), (ii) transporter associated with antigen processing (TAP)-dependent peptide transport (15), (iii) the affinity of the peptide for the major histocompatibility complex (MHC) molecule (6, 24), (iv) the rate of dissociation of the MHC molecule (26, 28), (v) the transport of MHC-peptide complexes to the cell surface (17), and (vi) the response to the T-cell repertoire (5, 7, 8).

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We have previously shown that intramuscular or gene gun immunizations with plasmid DNA encoding a single epitope of L. monocytogenes, LLO 91-99, are able to induce specific CTLs in a CD4+ T-cell-independent manner (19, 27, 31). In the current study, taking advantage of the fact that a single epitope-expressing DNA vaccine can induce CTLs without the influence of the other epitopes, we investigated the influence of dominant T-cell responses on subdominant responses.

We constructed three plasmids, p91m, p60 217m, and p60 449m, which encode LLO 91-99, p60 217-225, and p60 449-457 of L. monocytogenes, respectively, flanked by ATG start codon and TAA stop codon, under the control of the cytomegalovirus immediate-early enhancer-promoter. The DNA sequences inserted were adapted to the most frequently used codons in murine genes (19, 27). DNA immunization was performed into the shaved abdominal skin of BALB/c mice using the Helios gene gun system (Bio-Rad Laboratories, Hercules, Calif.). Then, 2 µg of plasmid DNA was coated onto 0.5 mg of 1.0-µm gold particles, and the injection was carried out with 0.5 mg of gold/shot. Mice were injected three times with 2 µg of the plasmids weekly. At 2 weeks after the last immunization, immune splenocytes were cultured in 12-well plates at a density of 2 × 10⁷/well for 5 days with 2 × 10⁷ syngeneic spleen cells per well that had been treated with 100 µg of mitomycin C per ml and pulsed with 5 µM concentrations of each of synthetic peptides representing LLO 91-99, p60 217-225, and p60 449-457 for 2 h at 37°C. Each well also received 10 U of human recombinant interleukin-2 (Hoffmann-La Roche, Nutley, N.J.) per ml. Cell-mediated cytotoxicity was assayed against J774 (H-2d) pulsed with incubation with 5 µM concentrations of each peptide using a conventional ⁵¹Cr release assay as described previously (27). As shown in Fig. 1A, gene gun immunizations of BALB/c mice with p91m and p60 217m expressing the dominant epitopes LLO 91-99 and p60 217-225, respectively, induced vigorous CTL responses, whereas immunizations with p60 449m encoding a subdominant epitope, p60 449-457, induced only a marginal CTL response. These CTL responses appeared to be the same as that observed with the immunization with live L. monocytogenes (4, 29). The results obtained
with nontant epitope, p60 449-457, has been reported to be associated with the three peptides expressed by the plasmids, since a subdominant CTL responses is attributable to quantitative difference of nternal antigens. It seems unlikely that the relative magnitudes of ntant and subdominant epitopes does not operate in the listerial epitopes. As shown in Fig. 2C, essentially the same results were obtained when mice were inoculated with gold particles coated with the mixed three molecules. The data obtained are essentially the same the results we observed with immunization with a single plasmid DNA, i.e., plasmids encoding epitopes versus plasmids encoding whole protein.

To investigate the effect of the in vivo interactions of T cells induced by the three different plasmid DNAs, p91m, p60 217m, and p60 449m, we immunized mice with the three different plasmid DNAs simultaneously at nonoverlapping sites of the abdominal skin and then evaluated the frequencies of T cells specific for LLO 91-99, p60 217-225, and p60 449-457 using ELISPOT (Fig. 2B). This method was designed to have different antigen-presenting cells present different epitopes to T cells in order to avoid the possibility of epitope competitions for binding to the same H-2Kd molecules. The data obtained are essentially the same the results we observed with immunization with a single plasmid DNA (Fig. 2A), suggesting that competitive interaction between CTLs against dominant and subdominant epitopes does not operate in the listerial epitopes. As shown in Fig. 2C, essentially the same results were obtained when mice were inoculated with gold particles coated with the mixed three plasmids (1 μg each). These data imply that the hierarchy of immunodominance in the listerial epitopes is readily determined by interaction between responder T cells and CTL epitopes presented by MHC class I molecules and that com-

imply that competitive interaction between CTLs against domin-

FIG. 1. Immunization with DNA vaccine encoding a single domin-
ant or subdominant epitope induces different levels of CTL activity. (A) BALB/c mice were immunized by gene gun with p91m ( ), p60 217m ( , ), or p60 449m ( , ) three times. Spleen cells from immunized animals were harvested 2 weeks after the last immuniza-
tion and stimulated in vitro with LLO 91-99-, p60 217-225-, or p60 449-457-peptide pulsed spleen cells for 5 days. The percentage of specific lysis was determined using 3774 cells (H-2d) pulsed with LLO 91-99-peptide (solid symbols) or control medium (open symbols) as target cells. Results are expressed as the mean ± the standard deviation (SD) for six mice. (B) Translational efficiencies of the three plasmid DNA vaccines. Relative luciferase activities were assayed by transient transfections of p91m-Luc, p60 217m-Luc, and p60 449m-Luc in BALB/3T3 mouse fibroblast cells. The relative luciferase activities are normalized to Renilla reniformis luciferase activities by the co-transfected pRL-CMV (Promega, Madison, Wis.). The data are expressed as the mean ± the SD for six samples.
petitive interaction between responder T cells may not operate in the generation of the hierarchy of the listerial epitopes. An attempt has been performed to delete the two dominant epitopes, LLO 91-99 and p60 217-225, from L. monocytogenes by anchor residue mutagenesis to study the influence in vivo of dominant T-cell response on the subdominant response during the listerial infection (30). Consistent with our data, the experiment demonstrated that the loss of these two dominant T-cell responses does not enhance T-cell responses to the subdominant epitopes (p60 449-457 and mpl 84-92).

Given that the three plasmid DNAs induced different levels of specific lysis, we wanted to determine the biologic effect of the plasmids against bacterial challenge. At 72 h after sublethal...
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REFERENCES


