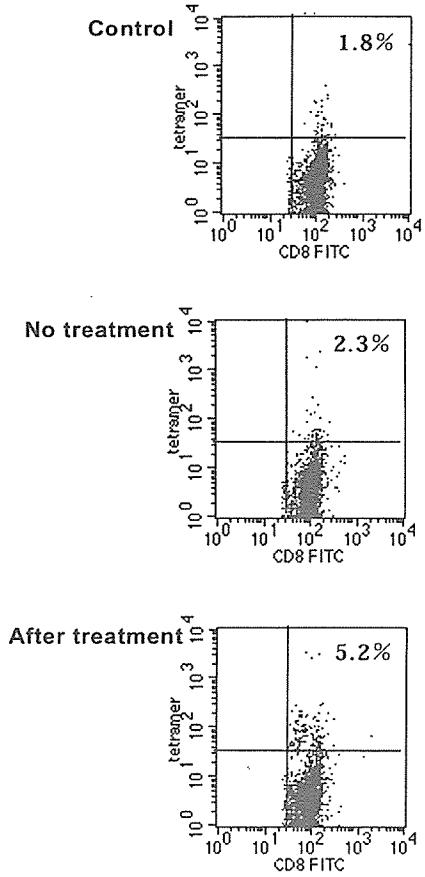


**FIGURE 5.** Induction of CTL by peptide-liposome conjugates inoculated in combination with CpG and anti-IL-10. CD8-gated cells were analyzed with a tetramer-detecting $OVA_{257-264}$ plus H-$2K^b$-specific T cells. Spleen cells of normal mice (Control), tumor-bearing mice without treatment (no treatment), or tumor-bearing mice that received inoculation with peptide-liposome conjugates in combination with CpG and anti-IL-10 (after treatment) were stained with PE-conjugated, tetramer-detecting $OVA_{257-264}$ plus H-$2K^b$-specific T cells and FITC-conjugated anti-CD8 Ab. The experiment was repeated three times with similar results.

combined inoculation with peptide-liposome conjugates, CpG, and anti-IL-10 Abs.

We have investigated (25, 39) the potential ability of surface-linked liposomal Ags for the application to vaccine development, whereas most of the investigations regarding liposomes as a drug-delivery system have been done by encapsulating Ags into liposomes. During the course of this investigation, several advantages of the liposome-coupled Ags over the liposome-encapsulated Ags became apparent. First, a predominant coupling efficiency of Ags to liposomes: following our previously reported procedure (20) for coupling Ags to liposomes, ~50% of the Ags bound to the surface of liposomes, whereas in the Ag encapsulation, a 60-fold higher volume of Ags was required to obtain the same amount of conjugates (our unpublished observation). Second, Ag-specific and IgE-selective unresponsiveness induced by surface-linked liposomal Ags: Ags chemically coupled to the surface of liposomes induced Ag-specific IgG but not IgE Ab production in mice (19) and also in monkeys (40), suggesting the potential ability of surface-linked liposomal Ags for application to the development of vaccines with minimal allergic side effects. In addition, during the course of an investigation intended to clarify the mechanism of IgE-selective unresponsiveness induced by surface-linked liposomal Ag, we found the existence of an alternative mechanism, not involving T cells, in the regulation of IgE synthesis (41). Third, an enhanced recognition of liposomal Ags by APCs: because liposomes basically consist of immunologically inert fatty acid, they are hardly recognized by APCs. Therefore, some contrivance, such as the introduction of mannose on the surface of liposomes (42), is required in Ag-encapsulated liposomes to enhance the recognition of liposomes by APCs. In contrast, in surface-linked liposomal Ags, Ags expressed on the surface of liposomes might be recognized more efficiently by APCs, which might result in an enhanced presentation to T cells. In fact, surface-linked liposomal Ags induced a significantly higher level of Ag-specific IgG production than that by liposome-encapsulated Ags in mice (our unpublished observation). In addition, a significant difference, which correlated closely with the adjuvant activity of liposomes, was observed in the recognition of liposomal Ags by APCs between liposomes with different lipid components; more Ags coupled to the unsaturated liposomes were engulfed by macrophages in vitro and a higher level of Ag-specific Ab production was induced in vivo than when saturated liposomes were used, suggesting that the adjuvant effects of liposomes are exerted at the beginning of the immune response, i.e., recognition of Ag by APCs (43). In addition to this quantitative difference between liposomes with differential lipid components, in the present study, a qualitative difference (i.e., the differential ability to induce cross-presentation) was observed between saturated and unsaturated liposomes. Although the precise mechanism underlying this difference is currently unclear, the significant difference in membrane mobility observed between these liposomes (23) might affect their ability to induce cross-presentation.

Because a detailed characterization of many tumor cell surface molecules that act as TAAs is now available (44), immunotherapy has become an increasingly essential component of cancer therapies (7). Emphasis to date has been placed on the development of cancer vaccines to enhance the immunogenicity of weak TAAs. In this context, surface-linked liposomal Ag might potentially serve as a candidate protocol for tumor vaccine preparation to present tumor Ags to APCs and induce effective antitumor responses.

## Disclosures

The authors have no financial conflict of interest.

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