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IL-10 Mediates Suppression of the CD8 T Cell IFN-γ Response to a Novel Viral Epitope in a Primed Host

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Priming to Ag can inhibit subsequent induction of an immune response to a new epitope incorporated into that Ag, a phenomenon referred to as original antigenic sin. In this study, we show that prior immunity to a virus capsid can inhibit subsequent induction of the IFN-γ effector T cell response to a novel CD8-restricted antigenic epitope associated with the virus capsid. Inhibition does not involve Ab to the virus capsid, as it is observed in animals lacking B cells. CD8-restricted virus-specific T cell responses are not required, as priming to virus without CTL induction is associated with inhibition. However, IL-10−/− mice, in contrast to IL-10+/+ mice, generate CD8 T cell and Ab responses to novel epitopes incorporated into a virus capsid, even when priming to the capsid has resulted in high titer Ab to the capsid. Furthermore, capsid-primed mice, unable to mount a response to a novel epitope in the capsid protein, are nevertheless able to respond to the same novel epitope delivered independently of the capsid. Thus, inhibition of responsiveness to a novel epitope in a virus-primed animal is a consequence of secretion of IL-10 in response to presented Ag, which inhibits local generation of new CD8 IFN-γ-secreting effector T cells. Induction of virus- or tumor Ag-specific CD8 effector T cells in the partially Ag-primed host may thus be facilitated by local neutralization of IL-10. The Journal of Immunology, 2003, 171: 4765–4772.

Papillomavirus (PV) major capsid protein L1 can self-assemble into virus-like particles (VLPs) when expressed in eukaryotic and prokaryotic systems (8–10). PV VLPs have been used to elicit high titers of systemic neutralizing Abs, which provide protection from experimental challenge with homologous infectious virus in animal PV models (11) and in humans (12). C-terminal truncation mutants of L1 protein also form VLPs, which can elicit immunity to PV and to CTL and B epitopes incorporated at the L1 C terminus, when administered systemically or mucosally (13–16). PV VLPs have thus been proposed as immunotherapeutic carrier systems (13, 15). However, human PV (HPV)-infected individuals develop serum Ab to VLPs (17). Recently, it has been proposed that pre-existing neutralizing Ab to VLPs contributes to the suppression of subsequent immune responses to incorporated Ag (18). In this study, we confirm that the CD8-restricted IFN-γ effector T cell response to an epitope incorporated into a PV VLP is inhibited by prior immunity to the VLP, and demonstrate, unexpectedly, that local secretion of IL-10 rather than pre-existing Ab is responsible for the inhibition.

Materials and Methods

Mice

Four- to eight-week-old adult female C57BL/6 mice and BALB/c mice were purchased specific pathogen free from the Animal Resource Centre (Perth, Australia); μMT C57BL/6 mice (19) were kindly provided by D. Tarlinton (Walter and Eliza Hall Institute, Victoria, Australia). IL-10−/− C57BL/6 mice were purchased from the Australian National University (Canberra, Australia). Mice were kept under specific pathogen-free conditions throughout, and all experiments were approved by and performed in compliance with the guidelines of the University of Queensland animal experimentation ethics committee.

1 This work was supported in part by Grants 142902, 142952, 252855, and 210248 from the National Health and Medical Research Council of Australia; the Queensland Cancer Fund; the Cancer Research Institute of New York; the Australian Cancer Research Foundation; and the Princess Alexandra Hospital Foundation.

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3 Abbreviations used in this paper: PV, papillomavirus; BPV, bovine PV; DTH, delayed-type hypersensitivity; HPV, human PV; LCMV, lymphocytic choriomeningitis virus; VLP, virus-like particle.
Cell lines and peptides

Sporobacteria frugiperda (SF-9) cells (Life Technologies, Rockville, MD) were maintained in SF-900 II medium with SF-9 II supplement (Life Technique) and 10% FBS (CSL, Melbourne, Australia) at 27°C. The peptide RAHYNIVTF, an H-2 b-restricted CTL epitope of residues 49–57 from the HPV16E7 protein (20); the peptide RGLPGRAVFT, an H-2 b-restricted CTL epitope of residues 318–327 from the HIVIIIB gp160 protein (21); and H-2 b-restricted CTL epitope of HPV11L1 YYHAGTSRL (22) were synthesized by Chiron Mimotopes (Melbourne, Australia). Recombinant HPV16E7gst protein was produced in the lab, as described elsewhere (23); and aluminum hydroxide gel adjuvant was purchased from Superfos Bio-sector (Vedbaek, Denmark). QuilA (Spikoside) (24) was purchased from Isotec (Lulea, Sweden). Mouse antitymoglobin was kindly provided by T. Stewart from the Center for Immunology and Cancer Research.

Construction of codon-modified HPV6bL1E7I plasmid DNA

Construction of a mammalian expression vector based on pcDNA3 and encoding a codon-modified 6bL1E7I gene, which comprises codon-modified HPV6bL1 truncated at the C-terminal 33 nt triplets fused to a gene encoding the first 50 aa of HPV6bE7, has been described elsewhere (25).

Production of rVLPs

VLPs were purified from the nuclei of SF-9 cells infected with L1 recombinant baculovirus by CsCl gradient centrifugation. VLPs with a density of 1.28 g/ml were collected and dialyzed extensively against PBS (13). Construction of baculovirus encoding HPV6bL1I, HPV11L1I, HPV16L1I, bovine PV1L1I (BPV1L1I), BPV1I/HIV1Ibp18I10CTL (p18VLPs), and BPV1I/HPV16E7ICTL (E7VLPs) recombinant protein has been described elsewhere (13, 26, 27). Samples were subjected to analysis by transmission electron microscopy and immunoblotting to confirm the identity and integrity of the VLPs. For immunoblot analysis, protein samples were diluted in SDS-PAGE sample buffer, boiled at 100°C for 10 min, electrophoresed through a 10% SDS-PAGE gel, and then transferred to nitrocellulose membrane. The membrane was blocked with 5% skim milk in PBS, and probed with the anti-L1 mAb MC15 (28) at a dilution of 1/2000. Bound Ab was detected by incubation of the membrane with HRP sheep anti-mouse Ab (Silenus, Melbourne, Australia) at a dilution of 1/10000 and visualized using ECL (Amersham, Arlington Heights, IL). For transmission electron microscopy, CsCl gradient-purified and dialyzed VLP samples were mounted onto carbon-coated grids, stained with 2% (w/v) uranyl acetate, and contrasted with 2% (w/v) lead citrate. Images were captured by a Gatan Orius microscope.

Immunization of mice

Groups of four or five mice were immunized as indicated with 30 or 50 μg of VLPs with or without alum. Mice were lightly anesthetized with Isoflurane (Abbott, Maidenhead, U.K.) during immunization. VLPs were in 50 μl of PBS or mixed with equal volume of alum or 10 μg QuilA. Immunization was performed on right hind leg, or left hind leg when separate immunization was needed. For production of specific antisera, C57BL/6 mice were immunized on days 0 and 14 with BPV L1VLPs or myoglobin (Sigma-Aldrich, St. Louis, MO) as an irrelevant Ag, and 7 days later, serum was collected and pooled, and inactivated at 56°C for 30 min. Anti-BPV L1I titters were measured by ELISA, as previously described (16).

DNA immunization

Immunization of mice with HPV6bL1 polynucleotide was undertaken, as described elsewhere (25). Briefly, plasmid was purified using a Qiagen Plasmid Mega Kit (Qiagen, Chatsworth, CA) and dissolved in PBS at a concentration of 1 μg/μl. C57BL/6 mice were immunized by particle bombardment with DNA-coated gold beads (2 μg DNA/dose) using the helium-powered Helios Gene Gun delivery system (Bio-Rad Laboratories, Richmond, CA). A total of 1 μg of DNA was coupled to 0.5 mg of 1.0-μm-diameter gold particles, as recommended by the manufacturer. DNA-coated microcarriers were delivered into the abdominal epidermis at a helium pressure setting of 400 psi. Mice were immunized three times at weekly intervals.

ELISA for VLP-specific total and subtype IgG Abs

Measurement of VLP-specific IgG Abs in serum was performed, as described (14). Briefly, serial dilutions of serum samples were added to poly- styrene microtiter plates (Maxisorp; Nunc, Roskilde, Denmark) coated with 50 μl of BPV1L1I. After incubation, HRP-conjugated goat anti-mouse IgG (Sigma-Aldrich) or HRP-conjugated goat anti-mouse IgG1 or IgG2a (Caltag, Burlingame, CA) was added. o-Phenylenediamine substrate was added, reaction was stopped with 3 N HCl, and plates were read at 495 nm using a Bio-Rad 4500 reader.

ELISA for IL-5, IFN-γ, and IL-10 cytokines from culture supernatants

A total of 1 ml of 2.5 × 106 splenocytes or pooled draining lymph nodes from mice immunized with 50 μg of E7VLPs with or without alum were cultured alone or in the presence of 10 μg/ml of E7VLPs for 48 h; supernatants were collected; and IL-5, IL-10, and IFN-γ were detected using ELISA kits from R&D Systems (Minneapolis, MN), performed according to the manufacturers’ instructions.

ELISPOT

This assay was performed, as described recently elsewhere (29). Briefly, single spleen cell suspensions were added to membrane-base 96-well plates (Millipore, Bedford, MA) coated with anti-IFN-γ (BD Pharmingen, San Diego, CA) with or without added IL-2 (Life Technologies) to each well. The relevant peptide Ag was added at various concentrations, and cells were held at 37°C with peptide overnight. Ag-specific IFN-γ-secreting cells were detected by sequential exposure of the plate to biotinylated anti-IFN-γ (BD Pharmingen), avidin-HRP (Sigma-Aldrich), and diaminobenzidine (Sigma-Aldrich).

Delayed-type hypersensitivity (DTH) testing

DTH reaction was assayed by an ear-swelling test in which 10 μl of purified VLPs (30) as the challenge Ag in 10 μl of 0.9% NaCl solution was introduced into the pinna of the ear, and ear thickness was compared at 48 h with the unchallenged ear, as previously described (31).

Results

The CD8 T cell IFN-γ response to a novel peptide epitope incorporated in a carrier Ag is impaired by prior immunity to the carrier Ag

To examine the effect of prior immunity to a particular viral carrier on the immune response to a novel epitope delivered with the carrier, mice were immunized with BPV1 L1VLPs, and were then immunized with BPV1 L1VLPs incorporating epitopes from HIV-1 gp120 protein (p18VLPs). Control mice were similarly immunized with p18VLPs without prior immunization with L1VLPs, and, as expected (13, 16), developed p18-specific T cell responses, as measured by ELISPOT. In contrast, the p18-specific response to p18VLPs was almost undetectable in mice immunized previously with L1VLPs (Fig 1A). To establish whether this observation could be generalized, a CD8-restricted epitope from the E7 protein of HPV16 was incorporated into BPV1 L1 to produce E7VLPs. The CD8 T cell IFN-γ response to this epitope following immunization with E7VLPs was compared between animals previously immunized with BPV1 L1VLPs, HPV6bL1VLPs, and naive animals. E7 peptide-specific IFN-γ-secreting T cells were, as expected, observed in mice immunized only with E7VLPs. They were absent in mice also immunized previously with L1VLPs, and present in mice also immunized previously with 6bL1VLPs (Fig 1B), showing that the impaired response was dependent on epitope sharing between the priming carrier Ag and the subsequently administered carrier Ag.

Ab to the carrier does not contribute to impaired induction of CD8 IFN-γ-secreting T cells by the haptenated carrier

A moderate degree of sequence conservation of L1 across PV genotypes suggested that cross-reactivity of Th cell responses might be expected to different PV L1 proteins. This was confirmed by comparison of the DTH response to different PV L1VLPs, in mice immunized with an HPV6 L1 polynucleotide vaccine (Fig 2). Th cell cross-reactivity has been similarly observed in humans, following immunization with HPV 11 VLP (32). These data, showing Th cross-reactivity between BPV-1 and HPV-6 VLPs, taken together with the data (Fig 1) that prior immunization with BPV-1
VLPs, but not HPV-6 VLPs, suppresses the E7 IFN-γ response generated by subsequent immunization with chimeric BPV1-E7VLPs suggest that suppression is unlikely to be attributable solely to a primed Th response. Ab responses to PV VLPs are, in contrast to Th responses, genotype specific, as predicted from genotype-associated hypervariability of VLP structure limited to the surface contact residues. These data suggested that VLP-specific Ab rather than Th cell responses might be involved in inhibition (18). To test this hypothesis, a number of experiments were undertaken. Serum from animals immunized with L1VLPs, or an irrelevant Ag, was passively transferred to recipient mice. The second day after passive transfer, serum anti-BPV1l1 reactivity was measured in recipients of L1VLP-specific antisera by ELISA, and the anti-BPV1l1 titer observed was comparable to that of pooled serum from L1VLP-immunized mice (Fig. 3A). Recipients of passively transferred Ab were then immunized with E7VLPs. Seven days after immunization, CD8 IFN-γ responses to the E7 epitope were assessed by ELISPOT, and no significant difference was observed between the responses following immunization with E7VLPs in the recipients of control or BPV1 L1VLP-specific serum (Fig. 3B). Similar lack of inhibition was observed when serum reactive with myoglobin was transferred to the recipients (Fig. 3C).

These data suggested that, in mice with prior immunity to a carrier VLP, factors other than pre-existing VLP-specific Ab or Th responses cause the inhibition of CD8 IFN-γ responses to a novel epitope subsequently delivered with the carrier VLP. To confirm this finding, BMT mice, which cannot produce Ab, were immunized with L1VLPs, and then with E7VLPs. Seven days after the second immunization, the CD8 T cell response to E7 was assessed by ELISPOT. As in naïve C57Bl/6 mice, E7-specific responses were induced in naïve BMT mice by E7VLPs (Fig. 4A), and, as in L1VLP-immunized C57Bl/6 mice, E7-specific responses could not be induced in primed BMT mice by E7VLPs (Fig. 4B). Thus, inhibition of CD8 T cell IFN-γ responses to a novel epitope in VLP-primed mice, is not achieved by and does not require VLP-specific Ab.

**Carrier priming inhibits induction of hapten-specific CD8 T cell IFN-γ responses even if the priming fails to induce carrierspecific CD8 T cells**

We next considered whether focusing of the CD8 T cell immune response in carrier-primed animals to dominant CD8-restricted epitopes in L1 could explain the observed lack of induction of a CD8 T cell response to a novel E7 epitope, in animals already immunized with L1VLPs. CTL epitopes have not been defined for BPV1 L1 protein, and in vitro assays for L1-specific CTL have not been developed, as overexpression of L1 protein in cells is generally lethal. We first demonstrated that VLP immunization with alum induces a Th2-type immune response. Mice immunized with E7VLPs with alum failed to generate E7-specific IFN-γ responses (Fig. 5A). The ratio of specific IgG2a to IgG1 in mice immunized with L1VLPs with or without alum (Fig. 5B) also shows that alum biases the L1VLP immune response to Th2 type. Immunization of E7VLPs with alum also induced a Th2-type cytokine profile, with increased production of IL-5, and decreased production of IFN-γ following in vitro Ag challenge, when compared with L1E7 immunization alone (Fig. 5, D and E). Similar results were obtained with cells from draining lymph nodes (data not shown). IL-10 secretion in response to VLP Ag was observed for cells from spleen or lymph node of animals immunized with VLPs with or without alum (Fig. 5F). To further confirm that alum adjuvant prevents induction by L1VLPs of an L1-specific IFN-γ response, BALB/c mice were immunized with HPV16 L1VLPs either alone

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**FIGURE 1.** Effect of priming with BPV1 L1VLPs (L1VLPs) on CTL reactivity to p18 peptide or to E7 peptide induced by immunization with L1VLPs incorporating these epitopes. BALB/c or C57Bl/6 mice (n = 5) were immunized on days 0 and 14 with 30 μg BPV1L1p18 (p18) VLPs or BPV1E7E7 (E7) VLPs without adjuvant. On days 21 and 35, mice were sacrificed at 7 days after the final immunization, spleens were pooled, and specific splenocyte IFN-γ responses were measured against p18 (A) and E7 (B) peptide (1 μg/ml) by ELISPOT. Results shown are the mean ± SEM of two independent experiments (A) or mean ± SEM of duplicates from one experiment (B).

**FIGURE 2.** Cross-reactivity of DTH responses between PV VLP of different genotypes. Mice were immunized with a codon-modified 6bL1 polynucleotide vaccine three times, and 7 days after the last immunization, mice were challenged with 10 μg of 6bL1VLPs, HPV11VLPs, HPV16VLPs, or L1VLPs, and ear thickness was compared at 48 h with the unchallenged ear.
or with alum, as an HPV16 L1 CTL epitope has recently been identified. IFN-γ responses to this L1 epitope were induced by HPV16 L1VLPs given without adjuvant, but were not observed in mice immunized with HPV16 L1VLPs with alum (Fig. 5C). Mice were therefore immunized with L1VLPs, with or without alum adjuvant, and then challenged with E7VLPs without alum adjuvant. No CD8 T cell IFN-γ response was seen to the E7 epitope following immunization with E7VLPs in mice primed with BPV L1VLPs, whether or not the L1VLPs were given with alum adjuvant (Fig. 5A).

These data suggest that inhibition of CD8 IFN-γ responses to novel epitopes associated with a carrier Ag following carrier priming is unlikely to be due to bias of the CD8 T cell response to already primed epitopes. To confirm that such inhibition could not be prevented by prior priming of the immunized animal to the relevant E7 epitope, mice were primed with HPV16E7, to generate CD8 T cells specific for this E7 epitope. These mice, and naive mice, were immunized with L1VLPs, and then E7VLPs as before. An IFN-γ response to the E7 epitope following immunization with E7VLPs was not observed, even in animals previously primed with E7 (Fig. 6). These results together suggest that functional dominance of L1 over E7 MHC class I-restricted epitopes in an L1-primed animal cannot explain the observed lack of E7-specific IFN-γ response to E7VLPs, in mice primed with L1VLPs.

Carrier priming does not inhibit induction of E7 epitope-specific CD8 IFN-γ T cells in the absence of IL-10

The data above demonstrate that immunization with L1VLPs does not need to induce VLP-specific Ab, primed B cells, or CD8 T cells to inhibit the CD8 T cell IFN-γ response to an E7 epitope incorporated into VLPs. We therefore hypothesized that cytokines released by primed T cells, upon recognition of L1 epitopes presented by APC following immunization with E7VLPs, might be locally inhibitory to induction of naive E7-specific IFN-γ-secreting CD8 T cells by the same or adjacent APCs. As IL-10 is a potent inhibitor of Ag presentation and T cell activation, IL-10 knockout mice, primed or not primed with L1VLPs, were tested for their ability to produce E7-specific CD8 T cells after immunization with E7VLPs. In IL-10−/− mice, but not in otherwise syngeneic IL-10+/− mice, E7-specific CD8 IFN-γ T cell responses were consistently observed in mice primed with L1VLPs, and subsequently immunized with E7VLPs (Fig 7A). The induced Ab to L1VLPs was similar in both groups (Fig. 7B), further confirming that VLP-specific Ab is not a determinant of the lack of E7 IFN-γ T cell response, following immunization of L1VLP-primed animals with E7VLPs. These data suggest that the inhibition of induction of E7-specific IFN-γ-secreting CD8 T cells was occurring

FIGURE 3. Passively transferred Ab to L1VLPs does not inhibit induction of immune responses to a novel epitope incorporated into the VLPs. A, C57BL/6 mice (n = 3) were injected i.p. with 1 ml of serum from mice immunized twice with L1VLPs. Two days after transfer, serum was collected and reactivity with BPV1 VLPs was measured by ELISA ( ) in comparison with serum reactivity in mice immunized twice with VLPs ( ). B, Recipients of passively transferred Ab ( ) and naive control mice ( ) were immunized with E7VLPs twice at 14-day interval. Naive mice were also immunized with HPV16E7 with QuilA ( ). Mice were sacrificed 7 days after the final immunization, and E7 peptide-specific IFN-γ responses were assessed for splenocytes by ELISPOT. The x-axis shows concentration of peptide (×10 μg/ml); y-axis represents the number of spots corresponding to IFN-γ-secreting cells. IL-2 indicates the addition of IL-2, but not peptide; M indicates both IL-2 and peptide were absent. Results are mean ± SEM of replicates of pooled splenocytes and represent one of two similar independent results. C, Mice recipient of passively transferred BPV-1-specific ( ) or myoglobin-specific ( ) mouse anti-serum, and control mice ( ) were subsequently immunized with E7VLPs and tested for E7-specific T cells by ELISPOT as for B.

FIGURE 4. Inhibition of E7VLP-induced E7-specific IFN-γ responses in animals previously immunized with L1VLP is also observed in μMT mice. A, μMT mice ( , n = 4) and C57BL/6 mice ( , n = 3) were immunized on days 0 and 14 with E7VLPs i.m. Unimmunized C57BL/6 mice ( , n = 3) were included as control. B, μMT mice ( , n = 8) and C57BL/6 mice ( , n = 4) were primed with L1VLPs on days 0 and 14, and then immunized with E7VLPs on days 28 and 35. As control, C57BL mice ( , n = 4) were immunized with E7VLPs twice on days 28 and 35, respectively. E7 peptide-specific splenocyte IFN-γ responses from individual mice were assessed by ELISPOT. Results are mean ± SEM of individual mice.
Inhibition of E7VLP-induced E7-specific responses following immunization with L1VLPs is observed whether or not immunization generates L1-specific IFN-γ responses. A, C57BL/6 mice (n = 4 per group) were immunized with L1VLPs with or without alum adjuvant on days 0 and 14. On days 21 and 35, mice were immunized with E7VLPs. Mice were immunized with E7VLPs with or without alum adjuvant on days 21 and 35 as control. Mice were sacrificed 7 days after the final immunization, and E7-specific responses were assessed for splenocytes by ELISPOT using E7 CTL peptide. B, IgG subtype of BPVL1 Ab response was measured by ELISA. C, BALB/c mice (n = 2) were immunized with 50 μg of HPV16L1VLPs with QuilA (●) or with alum (○) or 50 μg of OVA with alum (▼) twice on days 0 and 14, 7 days after the final immunization; IFN-γ responses to a HPV16L1 CTL1 epitope (YYHAGTSRL) were measured by ELISPOT. D–F, C57BL/6 mice (n = 6 per group) were immunized with E7VLPs with or without alum adjuvant on days 0 and 14. On day 21, splenocytes were collected and pulsed with 10 μg/ml E7VLPs for 48 h; supernatants were collected and assayed for IFN-γ (D), IL-5 (E), and IL-10 (F) by ELISA. Results shown are the mean ± SEM for cultures from individual mice.

Inhibition of E7VLP-induced E7-specific responses by prior immunization with L1VLPs occurs in E7-primed animals. C57BL/6 mice (n = 4) were immunized, as shown (A). In group G, the final E7 immunization was performed on the left hind leg muscle, while all other immunizations were on the right hind leg. Seven days after the final immunization, IFN-γ responses to E7 peptide were measured by ELISPOT. Results are mean ± SEM of duplicates and represent one of two similar independent experiments.

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E7 specific IFN-γ response spots per 10⁶ lymphocytes
by an IL-10-dependent mechanism local to the site of CTL induction. To confirm that this effect was local to the APC presenting the L1VLPs, animals immunized with L1VLPs and E7VLPs, which failed to generate E7-specific IFN-γ T cell responses, were shown to produce E7-specific T cell responses following immunization with E7 at a remote site (Fig 6), confirming that suppression of E7-specific CD8 IFN-γ responses by E7VLP, mediated by immunity to the carrier VLPs, involved local IL-10 production at the site of presentation of the L1 protein.

Discussion
In this study, it is demonstrated that the CD8 T cell IFN-γ response to a novel epitope, induced when animals are immunized with the epitope incorporated into PV VLPs as a carrier Ag, is markedly reduced by pre-existing immunity to the VLP. A reduced response requires IL-10 production, is local to the site of carrier protein delivery, and is independent of Ab to the carrier protein. These findings shed light on the immune mechanisms underlying the phenomenon referred to as original antigenic sin, commonly attributed to preformed Ab to the carrier protein, and also in particular the difficulty observed in inducing novel CD8-restricted IFN-γ immune responses to a tumor Ag, when an ineffective immune response to that tumor Ag is already present.

The observed inhibition of CTL response to an incorporated epitope that results from prior immunity to the carrier VLP fits to the phenomena of original antigenic sin, for which the immunologic basis remains incompletely defined. Original antigenic sin was first observed with influenza vaccine. Exposure to a second heterologous flu virus boosted Ab responses against the priming strain of the virus, with only modest responses directed against the heterologous virus (24, 33, 34). It has been shown that in the presence of anti-hapten Ab, memory B cells are preferentially activated in vivo by a structurally related haptenic analog for the priming hapten (35, 36). Memory B cell responses to a given Ag are dominant over naive B cell responses (37). It was suggested that memory B cells, generated during a prior exposure to a cross-reacting Ag, prevented or down-regulated the response to the unique new determinants on the Ag, possibly through differential effects of the FcγRI on the memory or naive B cell surface (3). More recently, CTL responses to lymphocytic choriomeningitis virus (LCMV) after infection with a variant LCMV in LCMV-infected animals were elicited mainly to determinants of the parental virus (38). Mice primed with a strain of wild-type LCMV (LCMV-WE) respond to a subsequent infection by LCMV-WE-derived CTL epitope variants with a CTL response directed against the initial epitope rather than against the new variant epitope.

One possibility considered for a lack of Ag-specific IFN-γ response to the novel incorporated epitope in the chimeric VLPs in the current study was therefore dominance of an L1 epitope over the novel epitopes. Epitope dominance in a primary immune response is held to be determined by the processing of Ag for presentation, or competition for loading into a limited number of cell surface MHC molecules between various peptide Ags (39–41). Both epitopes (p18 and E7 CTL epitope) chosen for incorporation in the present work are held to be dominant in their native proteins, and are among the dominant epitopes when delivered as a component of chimeric PV VLPs to a naive animal. In a secondary immune response to an Ag, pre-existing responses to a subset of the dominant epitopes can inhibit responses to other dominant epitopes (42). The dominance can be due to T cells competing for access to Ag-bearing APCs (43), i.e., L1 CTL epitope has the advantage of contact with APCs because of greater numbers and/or greater affinity. In the current model, when mice were primed with HPV16L1 or BPVL1 (E7) with alum, which prevents induction of E7 IFN-γ responses to both L1 and E7 (44) and induced a Th2-type cytokine environment with decreased IFN-γ, and increased IL-5 secretion in response to Ag exposure, inhibition of the response to the new epitope was nevertheless present. In another experiment, E7 IFN-γ response inhibition was still observed in mice that has been primed with E7, and that had strong E7 peptide-specific CD8-mediated IFN-γ responses before priming with L1VLP. These results indicate that prior induction of competing epitope clones is insufficient to explain the lack of E7 specific clones induced by E7VLPs in L1VLP-primed mice.

Ab has a suppressive effect on processing of T cell epitopes (45). It has been suggested that pre-existing Abs to HPV16L1 can suppress the E7 responses delivered by chimeric HPV16E7VLPs (18). However, we did not observe the same phenomenon in our model; passively transferred Ab or unrelated Ab did not suppress induction of IFN-γ responses to an incorporated epitope in the way that prior immunity to the VLP carrier did, despite achievement of comparable titers of Ab in the recipients of passively transferred serum. Also, inhibition of E7 IFN-γ response was still observed in μMT mice that did not produce Ab due to lack of B cells; furthermore, although IL-10 knockout mice developed Abs to L1VLP (Fig 7b), inhibition of the E7 IFN-γ response was not observed in these animals. Thus, from the data above, priming must induce suppression through activation of specific T cells and could not be attributed to pre-existing Ab. Contrasting observations by Da Silva et al. (18) were limited to demonstration that passive transfer of a VLP-specific immune serum inhibited generation of specific CD8 responses in the same way as prior VLP immunization, and might
reflect some properties of their immune system unrelated to VLP-specific Ab.

A population of regulatory T cells distinct from Th1 and Th2 cells has been demonstrated both in vivo and in vitro (46). These cells produce IL-10, but not IL-4 or IL-5, and are able to prevent autoimmune disease when adoptively transferred to otherwise susceptible hosts (47). T cells with this phenotype might therefore inhibit responses to E7 when E7 is presented by the same VLPs. Although IL-10 regulates growth and/or differentiation of CTLs and dendritic cells (48), it is unlikely that in our model circulating IL-10 inhibits E7 T cell responses directly, as the E7 response is not inhibited when E7 is delivered alone (Fig. 6). It is more likely that primed L1-specific Th cells, after contact with dendritic cells presenting L1 peptide, secrete IL-10, which locally alters DC function or specific T cell development, resulting in impaired induction of E7-specific immune responses (49). In vitro IL-10 secretion by splenocytes and lymph node lymphocytes (data not shown) in response to VLP Ag was easily detected in IL-10+/− animals immunized with VLPs, suggesting that this cytokine would be produced in significant amounts in vivo following presentation of chimeric L1-E7VLPs by APCs to potentially responsive naive E7-specific CD8 T cells and VLP-primed CD4 T cells in lymphoid tissues of VLP-primed animals.

Inhibition through priming is VLP genotype specific. Although we demonstrate in the current study that Th responses can be cross-reactive between different PV L1 proteins, as would be predicted by the >70% sequence homology between the different PV genotypes (50) and the relative promiscuity of MHC class II for Th peptides (32, 51), the observed lack of cross-inhibition between VLPs suggests that the demonstrated T cell cross-reactivity between PV genotypes may be of little biological relevance for induction of regulatory T cells. This observation is of some importance, as prior immunity to one PV genotype, induced by infection or prophylactic vaccination, would frequently be present in subjects infected with an unrelated PV genotype, and clearance of the new PV infection is thought to depend on induction of appropriate cell-mediated immune responses to PV protein epitopes not shared between PV types.

As immune responses to HPV infection are observed in HPV-infected patients, use of HPV VLPs as delivery system to induce CTL responses, as has been proposed, may be limited in those individuals previously exposed to PV virus. However, when mice primed with one type of PV VLPs were immunized with chimeric PV VLPs of a different PV genotype, inhibition of response to the new epitope was not observed, and thus use of VLPs of animal PVs may provide an alternative strategy to exploit the profoundly immunogenic nature of PV VLPs.

Taken together, our results indicate that pre-existing immunity to PV VLPs inhibits CTL responses induced to a novel antigenic epitope incorporated into subsequently delivered chimeric VLPs. Inhibition of CTL induction is IL-10 dependent, and local to the site of Ag presentation, and therefore local inhibition of IL-10 may be a key determinant of overcoming prior immunity to a carrier Ag when it is desired to induce a novel immune response through vaccination to a viral or tumor Ag.

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References


CORRECTIONS


The third author’s last name was misspelled. The correct spelling is Ruggiero.


In Results, the scale labels on the graphs in Figure 5C are incorrect. The labels for the x- and y-axes have been changed from “E7” to “L1” as shown in the revised figure below. The legend is correct as originally published.