Emergence of Regulatory CD4+ T Cell Response to Repetitive Stimulation with Antigen-Presenting Cells In Vitro: Implications in Designing Antigen-Presenting Cell-Based Tumor Vaccines

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Because APCs play a crucial role in the generation of T cell-mediated immune responses, numerous clinical trials with APC-based vaccines have been initiated in different types of human cancers. Encouraging results have emerged from some of these initial studies. Thus far, APC-based vaccinations usually include multiple rounds of immunization. With this approach, although we and others have detected induction of Ag-specific CTL responses in vaccinated patients after stimulation with the same APC-based immunogen, in vitro we also find that repetitive in vitro stimulation with Ag-loaded APC can, at times, lead to the emergence of noncytolytic CD4+ T cells exhibiting the characteristic phenotype of Th2 cells. These noncytolytic CD4+ T cells synthesize large quantities of type 2 cytokines such as IL-4 and IL-10 on stimulation with the autologous APC or tumor cells in an MHC class II-restricted manner. Further, these CD4+ T cells and a cell-free supernatant factor block the activation of fresh T lymphocytes. The supernatant factor also exhibits a marked inhibitory effect on the expression of the costimulatory molecules, CD80 and CD86, by APC. The inhibitory effect of the supernatant factor can be abrogated by neutralizing IL-10 in the supernatant. These observations therefore have implications in the APC-based tumor vaccine protocol design. The Journal of Immunology, 1999, 162: 5576–5583.

Antigen-presenting cells (APC) play a crucial role in the generation of T cell-mediated immune response (1). Several studies have shown that ex vivo-grown APC can be used in active specific vaccination schemes (2–4) to generate protective immunity in several tumor models. Understandably, these have renewed considerable interest in APC-based “cancer vaccine”. A number of “tumor-associated Ags” have now been structurally defined (5) and APC can be engineered, one way or another, to present a relevant “tumor Ag” (6). Thus, translational studies of surrogate cancer vaccine made with APC pulsed with specific peptide epitopes or with other forms of tumor Ag have been initiated in a number of laboratories. We and others have reported that immunizations with Ag (synthetic peptide, tumor cell fragments, or Id)-pulsed APC have biological activities in different human tumor systems (7–10). In this context, of considerable interest are the reports from Hsu et al. (8) and Nestle et al. (9) who have shown remarkable antitumor response with the Ag-pulsed APC-based immunization.

The results of the early translational studies provide a clear rationale for further studies designed to establish optimum protocols for immunization, to develop methods of patient monitoring, and to improve the efficacy of APC-based vaccine. Interestingly, most of the ongoing studies presently use multiple rounds of immunizations at fairly short intervals as the preferred method of immunization. Further, for patient monitoring, one of the popular approaches has been to look for T cells (derived from tumor nodules, vaccine sites, and circulation) capable of responding to the relevant Ag, in a suitable in vitro assay, after several rounds of specific stimulation. Indeed we and others (7, 9, 10) have been able to generate autologous tumor-reactive and peptide-specific CTL from the circulation, tumor deposits, and vaccine sites following in vitro stimulation with peptide-pulsed cultured APC. However, we also find that although in vitro stimulations of the effector cells lead to the generation of Ag-specific CTL, in vitro, repetitive stimulation with APC can at times lead to the emergence of noncytolytic CD4+ T cells exhibiting the characteristic phenotype of Th2 cells. Our studies further show that these CD4+ T cells and a cell-free supernatant factor derived from the stimulated CD4+ T cells are capable of blocking the activation of fresh lymphocytes when such lymphocytes are stimulated with lectin or by anti-CD3 Ab. A culture supernatant factor(s) from these CD4+ cells also exhibits a marked inhibitory effect on the expression of the costimulatory molecules, CD80 and CD86, by APCs. The inhibitory effect of the supernatant factor can, however, be almost totally abrogated by neutralizing the IL-10 activity in the supernatant. These observations therefore have implications in the design of APC-based tumor vaccine design.

Materials and Methods

Patients

Patients with metastatic melanoma and melanoma patients with no active disease but who were considered at risk of recurrence were entered into the...
Preparation of tumor lysate as a source of tumor-associated Ag

The procedure for preparation of the tumor cell lysate has been described (10). Fresh tumor tissues were homogenized without using any enzyme to obtain homogenates of single melanoma cells or melanoma cells in clumps. The resulting homogenate was washed and cell concentration was adjusted to $10^6$ cells/ml in PBS. A cellular lysate was then prepared from the homogenate by six repeated freezings (at $-80^\circ$C) and thawings to room temperature. Total lysis of all the cells was verified by trypan blue dye exclusion staining, cellular debris was removed by centrifugation (1000 x g for 10 min), and the protein concentration of the lysate was determined. Aliquots of the homogenates were then frozen at $-80^\circ$C for loading onto the cultured APC for vaccination or for in vitro assays.

Tissue culture

Tissue culture technique and the procedure of isolating fresh tumor cells from tissue explants have been described earlier (7, 11). Briefly, tissue cultures were performed in Iscove’s medium (Life Technologies, Gaithersburg, MD) supplemented with 10% FBS, t-arginine (0.55 mM), t-asparagine (0.24 mM), and t-glutamine (1.5 mM), henceforth described as complete medium (CM)(3). Fresh tumor cells were isolated by mechanical mincing of tumor tissues in serum-free medium.

APC culture

APC used in this study were peripheral myeloid APC expanded in vitro as described earlier (7, 10). Briefly, monocytes/macrophages were isolated as adherent cells from Ficoll-Hypaque gradient-derived mononuclear cell populations. The adherent cell were then cultured in CM containing 1000 U/ml of granulocyte-macrophage-CSF (Immunex, Seattle, WA) for 14 days. The nonadherent and loosely adherent cells were harvested by vigorous washing.

Peripheral blood lymphocytes

PBL were isolated on a Ficoll-Hypaque gradient (11). All cultures and experiments were performed in CM.

In vitro expansion of infiltrating lymphocytes

Lymphocytes infiltrating tumors (TIL) and lymphocytes infiltrating the vaccine site (VIL) were expanded, ex vivo, as previously described (7, 10). Briefly, homogenates of the excised tissues were prepared by mechanical means. The minced homogenates containing the infiltrating lymphocytes were then cultured in IL-2 (50 U/ml) in the presence of the appropriate immunogen (melanoma cell lysate-loaded or peptide-loaded autologous APC). The cultures were restimulated every 7 to 10 days with the respective immunogen. Phenotypic and functional analyses were performed as sufficient numbers of lymphocytes became available. All experiments shown in this communication were performed with the ex vivo-expanded infiltrating lymphocytes that were stimulated at least three times and were shown in this communication were performed with the ex vivo-expanded lymphocytes, in circulation and infiltrating excised vaccine sites and/or tumor sites, expanded in vitro in IL-2.

Preparation of tumor lysate as a source of tumor-associated Ag

The method for intracellular analysis of cytokine production by T cells has been described (13). Briefly, the effector cells (CD4+ T cells) are restimulated with the stimulator cells (APC) for 2 h (responder:stimulator ratio, 10:1) after which they are incubated with nonstimulatory doses of PMA (0.5 ng/ml) and ionomycin (4.0 ng/ml; Sigma, St. Louis, MO) for 4 h at 37°C. Brefeldin A (1 $\mu$g/ml; Sigma) is added 2 h before harvest. The cells are washed and stained with Cy-chrome-conjugated anti-CD4 (PharMingen, San Diego, CA) for 30 min on ice. They are then fixed in 4% paraformaldehyde (Sigma) for 25 min and permeabilized with PBS/BSA/saponin for 10 min. Cells were then stained with conjugated anti-cytokine mAb (IL-10) and analyzed on a FACScan, and the percentage of cytokine-producing cells within CD4+ cells are determined.

Results

Patterns of T cell response after ex vivo stimulation

One of our goals has been to establish long term lines of autologous melanoma-reactive and Ag-specific CTL from tumor sites, vaccine sites, and PBL from patients immunized with synthetic peptide-pulsed or melanoma lysate-loaded APC. Our approach was to present the relevant Ag (peptide or tumor lysate-pulsed APC) to autologous lymphocytes and expand the CTL populations with exogenous IL-2 through several rounds of weekly stimulation with the relevant immunogen. In several cases, we were able to detect the generation of CTL. Fig. 1 shows the results of functional characterization of several in vitro-expanded VIL and TIL populations, demonstrating their MHC class I-restricted and Ag-specific cytolytic activity. As shown, in one of these cases (RM), the VIL exhibited MHC class I-restricted lysis of the autologous melanoma cells (RM-M). The VIL also recognized the MAGE-1.A1 peptide, EADPTGHSY, quite selectively. Among the two TIL populations, TIL from RG recognized the autologous melanoma cells (JL-M) and another allogeneic melanoma cell line, GL-M. Of interest, both JL and GL were HLA-A2 positive. Although we clearly detected CTL activity in the cultured populations, we were unable to keep the Ag-specific CTL in culture for a prolonged period. In most cases, the CTL activities declined, at times precipitously, and a CD4+ noncytolytic population emerged.

Fig. 2 shows four experiments in four separate case studies of VIL or TIL as examples of the patterns of T cell responses observed following repeated in vitro stimulation with peptide-pulsed or tumor lysate-pulsed autologous APC or with autologous tumor. Fig. 2, a and b, shows one of these patterns characterized by a decline of the CTL activities (in one VIL and in one TIL population) in vitro cultures with concomitant rise of CD4+ T cells. In both cases, after 2 weeks of culture, there were more CD8+ T cells than CD4+ cells, which lysed the autologous melanoma cells in MHC class I-restricted fashion (as shown in Fig. 1). However, after several more rounds of stimulation, the CTL activities declined and a predominantly CD4+ population outgrew. Fig. 2c shows a different pattern of response in which the expanding T cells derived from a vaccine site became predominantly CD4+ from the beginning, showing very little cytolytic activity against...
the autologous melanoma cells. Fig. 2d shows the third pattern of T cell reactivity generated in in vitro stimulation of the TIL with autologous tumor cells. As shown, in this pattern, the CTL activity declined with time but the decline did not correspond with a fall of CD8\(^+\) T cells and concomitant rise of CD4\(^+\) noncytolytic T cells.

Functional analysis of ex vivo-expanded CD4\(^+\) T cells

We argued that the outgrowth of the CD4\(^+\) cells could have an effect on the decline of the CTL activity in these cultures. Accordingly, we undertook a detailed functional analyses of the CD4\(^+\) T cells emerging in these cultures. Indeed, when functionally analyzed, some of the CD4\(^+\) T cells emerging in culture after several rounds of stimulation showed all the characteristics of Th2 type cells. Table I shows the cytokine synthesis profile of the CD4\(^+\) T cells from the experiments outlined in Fig. 2, a and b. As shown, the CD4\(^+\) T cells from both cases synthesized IL-4 and IL-10 in a class II-restricted manner when they were restimulated with the autologous APC (Table I). Of considerable interest, the CD4\(^+\) T cells obtained from one of these cases (RG) recognized the autologous melanoma cells in a class II-restricted manner (Table I). The tumor cells in this case expressed HLA class II molecules (data not shown). Of further interest, the CD4\(^+\) T cells did not recognize allogeneic APC or allogeneic melanoma cells. The autologous melanoma cells RM-M or RG-M did not synthesize any detectable IL-10, suggesting that the IL-10 in these experiments was synthe-

sized by the T cells. Fig. 3 shows an intracytoplasmic cytokine analysis of the CD4\(^+\) T cells at single-cell level. As shown in the TIL, some of the CD4\(^+\) T cells synthesized IL-10 (Fig. 3b). However, a larger number of cells synthesized IL-10 (Fig. 3c) after stimulation with the autologous APC. Those CD4\(^+\) cells were found to secrete IL-10 are all CD3\(^+\) T lymphocytes (data not shown). Although the TIL culture used in this study contains predominantly CD4\(^+\) T cells, it also contains some CD3\(^+\) CD8\(^-\) T cells and some NK-like cells (total, ~25%), which might secrete IL-10 constitutively or due to the effect of PMA + ionomycin. Interestingly, these 25% of cells do not respond to further stimulation with APC (Fig. 3, b and c).

The emergence of Th2-type CD4\(^+\) T cell response in these type of cultures was not an isolated incidence; similar results were observed with all eight patients who received the peptide and APC-based vaccine (whether the cells were derived from PBL, VIL, or TIL). In fact, repeated in vitro stimulation of the PBL with the peptide-pulsed autologous APC ultimately led to the emergence of CD4\(^+\) Th2-type response. In four of these eight cases, CD4\(^+\) Th2 responses followed an initial CD8\(^+\) CTL response. VIL could be adequately expanded from only four of these eight cases. Three of these four expanded VIL eventually exhibited Th2-type CD4\(^+\) phenotype. In the group of patients in whom tumor lysate-loaded APC was used as vaccine (17 patients in total), VIL could be tested in detail in nine cases. The emergence of CD4\(^+\) Th2-type response was observed in four cases (10). Repeated stimulation of the PBL with Ag-loaded APC in these cases also led to the emergence of CD4\(^+\) Th2-type (collective data not shown).

Suppression of T cell activation by the expanded CD4\(^+\) T cells

The CD4\(^+\) T cells obtained from these cultures affected the activation of T cells (autologous or allogeneic) in in vitro coculture. PBL were stimulated in culture with 2 \(\mu\)g/ml PHA or with immobilized anti-CD3 Ab in the presence of these CD4\(^+\) T cells (PBL: CD4\(^+\) cell ratio, 10:1). Fig. 4 shows a representative experiment demonstrating that the proliferation of PBL was completely blocked in the presence of these cells. When culture supernatants collected from the PHA- or anti-CD3 Ab-stimulated PBL were analyzed for the presence of IL-2, it was observed that the production of IL-2 was significantly down-regulated in those cultures where this type of CD4\(^+\) T cell was added (Table II).

A number of experiments were conducted to examine the mechanism of suppression by these Th2-type CD4\(^+\) T cells. First, these experiments revealed that the culture supernatants collected from these cells were equally capable of inhibiting the suppressive activities. Table III shows an example of the suppressive effect of the culture supernatant from the CD4\(^+\) T cell culture. As shown, the culture supernatant added to the fresh cultures of T cells blocked T cell activation with different stimuli. Interestingly, the supernatant factor also had a strong negative effect on the up-regulation of the expression of the costimulatory molecules CD80 and CD86 by the APC (data not shown). This type of inhibitory effect of the culture supernatants on the expression of the costimulatory molecules (CD80 and CD86) on the APC was seen in all three of three cases studied (collective data not shown).

Effect of neutralization of IL-10 on CTL activity in a long term culture

Since CD4\(^+\) T cells outgrowing in these cultures synthesized type 2 cytokine and because IL-10 has been known to possess considerable inhibitory activity on APC and T cells, we examined the effect of neutralization of IL-10 on the maintenance of CTL activity using one of our patients who was immunized with the MAGE-3.A2 peptide (FLWGPRALV)-pulsed APC in whom we
FIGURE 2. Pattern of in vitro T cell response from vaccine site or tumor tissue following repetitive stimulation. a, Phenotype and cytotoxic function of VIL from patient RM; b, phenotype and cytotoxic function of TIL from patient RG; c, phenotype and cytotoxic function of VIL from patient RP; d, phenotype and cytotoxic function of TIL from patient. □, T4; ▲, T8; ●, cell-mediated cytotoxicity.

Table I. Cytokine synthesis profile of the CD4+ T cells outgrowing in two culture systems

<table>
<thead>
<tr>
<th>Stimulated by</th>
<th>IL-2 (U/ml)</th>
<th>IFN-γ (pg/ml)</th>
<th>TNF-α (pg/ml)</th>
<th>IL-4 (pg/ml)</th>
<th>IL-10 (pg/ml)</th>
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</thead>
<tbody>
<tr>
<td>VIL (from RM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0.0</td>
<td>4</td>
<td>0</td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td>APC alone</td>
<td>0.90</td>
<td>15</td>
<td>35</td>
<td>1500</td>
<td>600</td>
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<tr>
<td>APC + anti-MHC class I Ab</td>
<td>1400</td>
<td>500</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APC + anti-MHC class II Ab</td>
<td>400</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
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<td>Allogeneic APC 1</td>
<td>120</td>
<td>10</td>
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<td></td>
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<tr>
<td>Allogeneic APC 2</td>
<td>50</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Autologous melanoma line</td>
<td>40</td>
<td>40</td>
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<td>TIL (from RG)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>40</td>
<td>42</td>
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<tr>
<td>Autologous APC</td>
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<td>0</td>
<td>0</td>
<td>1250</td>
<td>500</td>
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<tr>
<td>Autologous APC + anti-MHC class I Ab</td>
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<td>500</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autologous APC + anti-MHC class II Ab</td>
<td>60</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Allogeneic APC</td>
<td>76</td>
<td>70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autologous melanoma cells+</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>1000</td>
<td>400</td>
</tr>
<tr>
<td>Autologous melanoma cells + anti-MHC class I Ab</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>1100</td>
<td>350</td>
</tr>
<tr>
<td>Autologous melanoma cells + anti-MHC class II Ab</td>
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<td>54</td>
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<td>Allogeneic melanoma line</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>42</td>
<td>50</td>
</tr>
</tbody>
</table>

* The VIL/TIL were expanded in exogenous IL-2 with four weekly stimulations by MAGE-1.A1 nonapeptide (EADPTGHSY) for VIL or by MAGE-3.A2 nonapeptide (FLWQPRALV) for TIL, pulsed autologous APC. The expanded cells were stimulated, and culture supernatants harvested 24 h were assayed for the cytokines.

* In this case, autologous melanoma cells expressed MHC-II moleculars.
observed the rise and decline of CTL activity with concomitant rise of CD4\(^+\) T cells in the TIL culture (shown earlier in Fig. 2b). Fig. 5 shows the result of the experiment. The TIL from this case were cultured in IL-2 medium in the absence or in the continuous presence of anti-IL-4, anti-IL-10, or control mouse Ab for 60 days. The cultures were restimulated every 10–12 days. As shown, the CTL activity declined within 2 wk in the standard culture. However, the neutralization of IL-10 (and not IL-4) maintained the CTL activity up to 60 days. Similarly, when the PBL from the same case were stimulated with the MAGE-3.A2 peptide (FLWGPRALV)-pulsed APC in the presence of anti-IL-10 Ab, peptide-specific and autologous melanoma-reactive CTL activity could also be generated and maintained for 60 days (Table IV). The neutralization of IL-10 prevented the decline of CD8\(^+\) T cells in the continuous culture and helped maintain their continued growth (data not shown).

**Discussion**

One of the lessons learned from the studies of T cell-determined "melanoma Ags" is that all self-reactive T cells are not centrally deleted. The current interest in active specific immunization in human cancer with “tumor associated self Ags,” defined or undefined, derives its reason for existence from the very fact that T cells potentially recruitable against these types of determinants exist and that tumor cells express some of these determinants, although not necessarily selectively. These basic observations have led to several approaches to developing novel cancer vaccines. Among them, immunization with a relevant synthetic T cell-determined peptide, with or without a suitable adjuvant, and immunization with Ag-loaded APC (usually dendritic cells) have gained much popularity. Evidence of biological activity of both forms of “cancer vaccine” has already emerged (7–10, 14–17). Collectively, these observations provide considerable support for these types of approaches. However, further refinements will be needed to make these approaches more effective. In this context, we believe that a better understanding of the mechanisms of peripheral tolerance to these types of “self” determinants will be helpful. Methods could then be devised to circumvent physiological constraints against unleashing a potentially self-injurious immune reaction, especially when dealing with a “self” Ag. Traditional views of peripheral tolerance to these types of self Ags are that they are due to “anergy” (18), exhaustion (19), ignorance (20, 21), peripheral deletion by apoptosis of T cells through a FAS/FAS-L interaction (22), or a variety of immunosuppressive cytokines that cancer cells can...
elaborate. When the relevant T cells are not deleted yet remain in a “tolerant” state, it is possible to argue that the success of immunization with tumor-associated Ags, which are essentially self Ags, might depend on the balance between the capacity of the immunogenic stimulus to “break tolerance” and on the inherent physiological constraint against activation of “self” reactive T cells.

In this equation, suppression of T cell response that might have been induced spontaneously (as in autoimmunity) or deliberately (as in immunotherapy) cannot be ignored, especially when the T cell response is induced against self Ag. The concept of T cell-mediated suppression of cellular immune response has been controversial. However, a functional dichotomy with CD4+ T cells into Th1 and Th2 types exhibiting opposite effects (Th2 type acting as regulatory cells in some situations) is fairly well established (23). Indeed, in some animal models, the ultimate nature of the immune response to certain types of infection (i.e., protection vs infection) is often determined by whether the animal mounts a Th1- or Th2-type response (24). Thus, the observations reported here are noteworthy. First, the decline of CTL response, in vitro, despite antigenic stimulation provided through APC expressing all the essential costimulatory molecules and the emergence of the Th2 type CD4+ T cells, suggests that the window of opportunity to expand a CTL response to these types of self Ag might be quite narrow. Second, the emergence of the CD4+Th2-type response following a CTL response might represent a form of inherent regulatory mechanism for controlling harmful expansion of “self-reactive” T cells. Third, the mechanism of regulation by these CD4+ T cells, as well as their target, is quite interesting. These CD4+ T cells suppress T cell activation directly as evidenced by their suppressive effect on the lectin- or TCR-driven activation of fresh T cells (Table III). This is in line with our earlier study in which we have shown that CD4+-regulatory cells can exert an inhibitory effect on the T cells by down-regulating the expression of IL-2 receptor (25, 26). We and other investigators have found that in addition to affecting T cell activation directly, these types of CD4+ T cells can also down-regulate the expression of costimulatory molecules on the APC (collective data not shown (27)). Although we have not directly addressed the issue, it is possible to argue that by down-regulating the expression of costimulatory molecules on the APC, CD4+ T cells can render the APC ineffective as APCs and/or as stimulatory cells. This type of CD4+ T cells, therefore, seems capable of exerting a much broader regulatory effect, resulting in the suppression of T cell activation. In this way, CD4+ T cells seem to have a foolproof inhibitory effect on the generation of T cell-mediated immune response. Fourth, it seems that this type of CD4+ T cells can exert their regulatory function through a cytokine that appears to be IL-10 (Table IV and Fig. 5), although several reports have indicated that this type of suppressive effect could in part be attributed to TGF-β secreted by the regulatory T cells (30). Admittedly, the relationship between the different subsets of regulatory T cells is complex and unclear at present. In our present observation, indeed, neutralization of IL-10 in the cultures maintained the CTL activity for a considerable length of time (Table IV and Fig. 5). In this context, it should be pointed out that IL-10 has been a remarkable immunoregulatory cytokine. Although some investigators have found IL-10 to have a “stimulatory” effect (28, 29), the great majority of the published work attests to its inhibitory effect on APC, T cells, and NK cells (27, 31–36).

The physiological relevance of these types of CD4+ T cells emerging in culture is admittedly unclear. The outgrowth of CD4+ T cells in our system might have resulted from their inherent proliferative advantage in in vitro culture containing exogenous IL-2. However, an extensive literature on the negative role of CD4+ T cells in tumor immunity exists (37, 38). Ag-specific, as well as Ag nonspecific, suppressor CD4+ T cells have been described (37, 38, 42). In contrast, evidence of beneficial effect of removal of CD4+ T cells in context to tumor immune manipulations has also been described by several investigators (39–41). Thus, the emergence of this type of cells might have physiological relevance. Of interest, a profound inhibitory function of CD4+ T cells, in vitro and in vivo, has recently been documented by several different groups of investigators in two murine disease models (43–45). Although it is not possible to conclude that repeated immunization with APC-based vaccine in vivo might have a similar inhibitory effect, it is
not inconceivable that repeated APC-based vaccination might facilitate the emergence of the process as a countermeasure to expansion of potentially injurious self-reactive T cell populations. This could be an Achilles heel in the currently ongoing APC-based cancer immunotherapy. Controversies will undoubtedly continue on the subject of T cell-mediated suppression of immune response. It should be pointed out, however, that in context to vaccine therapy for cancer, opportunities exist to test a number of approaches designed to widen the window of opportunity of expanding tumor-associated but self Ag-specific CTL and to circumvent this type of potentially negative role of Th2-type response. Among others, these approaches could include: a different vaccination protocol, such as quick priming, followed by periodic booster injections; the use of pharmacological inhibitors of Th2-type response; the inclusion of a Th1-type cytokine such as IL-12; or the engineering of APC to synthesize Th1-type cytokine, such as IL-12, locally.

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References


