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*J Immunol* 2009; 182:6095-6104; doi: 10.4049/jimmunol.0803829
http://www.jimmunol.org/content/182/10/6095

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Effector/Memory but Not Naive Regulatory T Cells Are Responsible for the Loss of Concomitant Tumor Immunity

Yung-Chang Lin,*‡ Li-Yuan Chang,† Ching-Tai Huang,*¶ Hui-Min Peng,§ Avijit Dutta,¶ Tse-Ching Chen,*∥ Chau-Ting Yeh,¶∥ and Chun-Yen Lin²§¶

The phenomenon of concomitant tumor immunity involves a tumor-bearing host rejecting another similar tumor at a distant site and suggests the existence of tumor-specific immunity. Loss of this immunity may contribute to tumor metastasis. Nevertheless, underlying mechanisms for concomitant tumor immunity loss are still unknown.

Concomitant tumor immunity, first reported by Ehrlich (1) and Bashford et al. (2), is a phenomenon in which an immunogenic tumor-bearing host rejects inoculums of a similar tumor at a distant site, in a wide variety of tumor models (1–5). Nevertheless, a few investigators have found this response to be transient, as the immunity can decay as the primary tumor progresses. This phenomenon has been deemed a mechanism for tumor metastasis (4–6). In a series of elegant studies, it was proposed that concomitant tumor immunity represents a balance between tumor-induced CD8+ effectors and suppressor T cells (7, 8). However, after this proposal, there was no progress made in the area for two decades, as the existence of suppressor cells was in doubt. Recent interest in suppressor T cells has led to recognition of regulatory T (Treg) cells, which have been shown to be the major regulators responsible for weak concomitant tumor immunity against some immunogenically inert tumors (9). However, underlying mechanisms for concomitant tumor immunity loss are still unknown.

Conversely, the critical role of Treg cells in tumor immunotherapy has been gradually elucidated (10). Treg cells (defined as CD4+CD25+Foxp3+ T cells) have been shown to be inversely related to the outcomes of several human malignancies (11–13). Furthermore, depletion of Treg cells can enhance rejection of endogenous immune-mediated tumor and improve tumor Ag-specific immunity, which highlights the impact of Treg cells on antitumor immunity (14, 15). Depletion of Treg cells has also been shown to improve the efficacy of tumor immunotherapy, including vaccination and CTLA4 blockade (16, 17). Treg cells also inhibit effector functions of both tumor-specific CD8+ and CD4+ T cells through a partially understood mechanism involving cell-cell contact or production of soluble factors such as IL-10 or TGF-β (18–20).

Recently, Treg cells have been subgrouped as either naive or effector/memory by their developmental stage and phenotype (21, 22). Effector/memory Treg cells are characterized by CD103 expression and the ability to home into inflammation sites (22). They have been proposed to be sentinels of tolerance, providing a first line of defense against potentially harmful inflammatory reactions (21). Treg cells have also been found in tumor tissue and as components of tumor-infiltrating lymphocytes (TILs) (23). Furthermore, the ratio of CD8+ T cells to Treg cells in TILs is highly correlated with outcomes of ovarian cancer patients (24). This correlation implies that tumor tissue might be a venue in which Treg cells impose inhibition of tumor-specific CD8+ T cells. Interestingly, tumors have also been deemed to be inflamed tissue (25). Some evidence suggests that oncoeneses initiate an inflammatory program highly associated with tumor invasiveness and metastasis (26, 27). However, the relationship with tumors, antitumor immunity, and effector/memory Treg cells is unexplored.

We set up a murine concomitant tumor immunity model using a CT 26 colon cancer cell line. In this model, we demonstrate that concomitant tumor immunity gradually decayed as the primary tumor progressed. Despite this loss of immunity, CD8+ T cells harvested from these mice still had potent antitumor immunity. During this period, effector/memory Treg cell counts increased not only in the TILs but also in the spleens of these mice. These cells
showed greater expression of effector molecules, including perforin, granzyme B, and inflammation-related chemokine receptors. In addition, using adoptive transfer, we clearly show that these effector/memory Treg cells lead to the loss of concomitant tumor immunity associated with tumor progress. The present study indicates that effector/memory Treg cells not only inhibit immune responses for tissue protection, as in other diseases like graft-vs-host disease and chronic infection, but also protect the tumor from immune attack (28, 29). These results clarify the relationship between tumors, inflammation, and effector/memory Treg cells.

Materials and Methods

Mice

BALB/c mice, purchased from the National Science Council of Taiwan (Taipei, Taiwan), were bred in the animal house of Chang Gung Memorial Hospital (Taoyuan, Taiwan) and were used in our experiment at ages 8–10 wk. All animal breeding and experiments were in accordance with guidelines of the institutional animal ethics committee.

Tumor model establishment

The murine (BALB/c) colon cancer cell line, CT 26, was obtained from the American Type Culture Collection (Union Biomed) and was maintained in our laboratory. Cells were regularly cultured in vitro in RPMI 1640 medium containing 10% FBS. A total of 1 × 10^5 live CT 26 cells (viability >95%) were harvested, washed in HBSS, and inoculated s.c. into the left flanks of naive mice (primary tumor). In cases of secondary challenge (secondary tumor), 1 × 10^5 live CT 26 cells were inoculated into the contralateral sides of mice that received primary injections. Tumor diameters were measured bidirectionally, twice a week.

Ab and flow cytometry

The following Abs were used for flow cytometry staining: PerCP-conjugated anti-CD4 (RM-4-5), PE- or FITC-conjugated anti-CD8 (53-6.7), PE- or FITC-conjugated anti-CD4 (RM4-5), PE- or FITC-conjugated anti-CD103 (M290), allophycocyanin-conjugated anti-CD62L (MEL-14), FITC-conjugated anti-CCR7 (C53-34-448), biotin-conjugated anti-Fas ligand (anti-FasL, MFL3), PE-conjugated anti-glucocorticoid-induced TNFR (anti-GITR, DTA-1), PE-conjugated anti-CTLA4 (UC10-4F10-11), bispecific anti-ICOS (7E.17G9), and streptavidin-allophycocyanin-conjugated anti-ICOS (7E.17G9), and allophycocyanin-conjugated anti-ICOS (7E.17G9), and allophycocyanin-conjugated anti-Foxp3 (FJK-16s), isotype control rat IgG2a and rat IgG2b, all purchased from eBioscience. PE-conjugated anti-CCR3 (83101) was purchased from R&D Systems. Single-cell suspensions were incubated with appropriate Abs for staining per the manufacturer’s instructions. Stained cells were resuspended in PBS and analyzed by flow cytometry.

Cell purification and adoptive transfer

Donor mice were sacrificed and their spleens were harvested under sterile conditions. Single-cell suspensions were prepared and CD8^+ T cells were isolated using magnetic microbeads conjugated with anti-mouse CD8 (Miltenyi Biotec) by AutoMACS (Miltenyi Biotec), according to the manufacturer’s instructions. For CD4^+ CD25^−, CD4^+ CD25^+, and CD4^+ CD25^+ CD103^+ T cell purification, splenocytes were first enriched for CD4^+ T cells via negative selection using the CD4 isolation kit (Miltenyi Biotec). Enriched CD4^+ T cells were then labeled with CD25-PE and CD103-FITC and sorted using FACSaria (BD Biosciences). Cell purity (>90%) for all populations was confirmed by flow cytometry. Different isolated populations of T cells were adoptively transferred into mice or used in vitro. For adoptive transfer, purified T cells in appropriate numbers were resuspended in 0.2 ml of HBSS and then injected i.v. into different mice through the tail vein.

Isolation of TILs

Tumors were chopped into small pieces using a razor blade and incubated with collagenase D (0.1%; Sigma-Aldrich) in HBSS for 30 min at 37°C. After passing through nylon mesh, single-cell suspension was separated with Ficoll (Pharmacia), and leukocytes were recovered from the interphase.

In vitro cytokine production assay

Whole splenocytes or specific purified populations of splenic T cells (5 × 10^5 cells/ml) from experimental mice were restimulated in vitro by culturing them with irradiated CT 26 parental tumor cells (5 × 10^5 cells/ml or 5 × 10^4 cells/ml) for 48 h. Supernatants were collected and assayed for IFN-γ production by ELISA according to manufacturer instructions (BD Biosciences).

Statistical analysis

Mann-Whitney U test was used for statistical analyses of differences between groups. The calculations were made using Prism (version 5.00; GraphPad Software). Differences were recognized as significant at p < 0.05.

Results

Loss of concomitant tumor immunity paralleled tumor progression

In line with previous literature involving diverse tumor systems, concomitant tumor immunity was iterated in our CT 26 colon cancer cell line induced-tumor model in BALB/c mice (1–10). As shown in Fig. 1A, the growth of secondary-inoculated CT 26 tumor cells (secondary tumor) in the contralateral side was prevented when they were injected s.c. 7 days after the first CT 26 tumor challenge (primary tumor). Interestingly, the primary tumor growth pattern was the same, regardless of secondary tumor inoculation presence or absence (Fig. 1A).

To explore this phenomenon, mice were then challenged with the secondary CT 26 tumor on different days after primary CT26 tumor inoculation (Fig. 1B). As shown in Fig. 1C, the secondary inoculum of CT 26 escaped immune suppression if it was inoculated 14 days after primary tumor challenge; concomitant tumor immunity almost disappeared when secondary inoculation was done 28 days after primary inoculation (Fig. 1C). These results demonstrate that concomitant tumor immunity decreases gradually with time as well as with primary tumor progression.

Competent tumor-inhibiting ability of CD8^+ T cells from mice that lost concomitant tumor immunity

The next issue we addressed was the underlying cellular mechanism for concomitant tumor immunity loss. Splenocytes from mice that lost concomitant tumor immunity (day-28 tumor-bearing mice) were adoptively transferred to another group of mice that received first CT 26 cell tumor inoculation 6 days before. On day 7 (the next day after splenocyte transfer), the secondary inoculum was challenged to each mouse. Results show that splenocytes from day-28 tumor-bearing mice (day-28 splenocytes) could not reverse the concomitant tumor immunity but they did decrease the primary tumor growth rate (Fig. 2A). In addition, the CT 26-specific IFN-γ responses of these day-28 splenocytes were similar to the splenocytes of day-7 primary tumor-bearing mice (day-7 splenocytes) and may be responsible for the concomitant tumor immunity (Fig. 2B). These results indicate that mice, even after the loss of concomitant tumor immunity, retain substantial tumor-inhibiting ability.

We fractioned day-28 splenocytes to study the impact of different T cell populations, from day-28 tumor-bearing mice, on tumor growth. Observations suggest that CD8^+ T cells from day-28...
splenocytes partially retard primary tumor growth (Fig. 2C), whereas CD4+ T cells from day-28 splenocytes inhibit concomitant tumor immunity (Fig. 2D).

It is intriguing that CD8+ T cells from mice that lose concomitant tumor immunity can restrain tumor growth. We investigated phenotype changes in CD8+ T cells in different compartments of tumor-bearing mice. As shown in Fig. 3A, the percentage of activated CD8+ T cells (defined as CD25+, CD44high, or CD69+) increased mainly in draining lymph nodes and the spleen but less in nondraining lymph nodes of tumor-bearing mice when compared with naive mice. CD8+ T cells in TILs were also highly activated and showed strong effector molecule expression, including FasL, perforin, and granzyme B (Fig. 3B). Furthermore, the percentage of these activated and functionally mature CD8+ T cells was higher in day-28 tumor-bearing mice than day-7 tumor-bearing mice (Fig. 3B).
To corroborate our findings concerning functional capabilities of CD8+ T cells in vitro and in vivo, these cells were sorted from day-7 and day-28 spleens of tumor-bearing mice; in vitro CT 26-specific IFN-γ secretion was significantly enhanced in CD8+ T cells of day-28 spleens, rather than day-7 spleens or naive CD8+ T cells (Fig. 3C). The in vivo tumor-inhibiting ability of cells was then evaluated by adoptively transferring these cells to naive mice, 1 day after primary CT 26 tumor challenge. As shown in Fig. 3D, CD8+ T cells from day-28 spleens could inhibit the growth of tumor in a dose-dependent manner. Interestingly, CD8+ T cells from day-28 spleens were the most potent in inhibiting tumor growth when compared with CD8+ T cells from day-7 spleens or naive CD8+ T cells (Fig. 3E). Taken together, these results demonstrate that the CD8+ T cells that lost concomitant tumor immunity retained tumor-inhibiting potency.

**CD4+ CD25+ Foxp3+ T cells from tumor-bearing mice can reduce tumor-inhibiting potency of CD8+ T cells**

It seems that CD8+ T cells from tumor-bearing mice are potent inhibitors of primary tumor growth, but why do these mice lose concomitant tumor immunity? We enumerated Treg cells (CD4+ CD25+ Foxp3+ T cells) in different areas of tumor-bearing mice and compared them with those from naive mice. The absolute number of Treg cells were increased in the spleen and draining lymph nodes but not in non-draining lymph nodes in tumor-bearing mice (Fig. 4A). We sorted CD8+ T cells (day-28 CD8+ T cells), CD4+ CD25+ T cells (day-28 CD4+ CD25+ T cells) and CD4+ CD25+ T cells (day-28 Treg cells) from day-28 tumor-bearing mice spleens. As shown in Fig. 4B, the day-28 CD4+ CD25+ T cells had inhibitory capability, whereas day-28 CD4+ CD25+ T cells had no additional effect on day-28 CD8+ T cells in terms of IFN-γ secretion. We cotransferred day-28 Treg cells or day-28 CD4+ CD25+ T cells with day-28 CD8+ T cells into naive mice and then challenged the mice with CT 26 tumor cells. As shown in Fig. 4C, the day-28 Treg cells but not the day-28 CD4+ CD25+ T cells could reverse day-28 CD8+ T cell-mediated primary tumor suppression.

We further compared the suppression ability of naive Treg cells and day-28 Treg cells. The data show the suppression effects of day-28 Treg cells on the in vitro tumor-specific IFN-γ secretion of...
FIGURE 3. Competent tumor-eradication potency of CD8^+ T cells from mice that have lost concomitant tumor immunity. A, Expression of activation markers (CD25, CD44, CD69) on CD8^+ T cells from immune organs of naive mice, day-7 or day-28 tumor-bearing mice. B, Expression profile of activation markers and effector molecules (CD25, CD44, CD69, FasL, perforin, granzyme B) of tumor-infiltrated CD8^+ T cells (TIL) from day-7 and day-28 tumor-bearing mice. C, Estimation of IFN-γ production by CD8^+ T cells purified through AutoMACS from spleens of naive mice, 7-day or 28-day tumor-bearing mice after stimulation with different doses of irradiated CT 26 cells by ELISA. D, On day 0, 1 x 10^5 CT 26 cells were inoculated. On day 1, no cell transfer (group A) or with a different number of splenic CD8^+ T cells (group B, 0.3 x 10^6; group C, 1 x 10^6; and group D, 3 x 10^6) purified from day-28 tumor-bearing mice were i.v. injected. Growth patterns of each group were measured. E, CD8^+ T cells (1 x 10^6 per mice) from naive, 7-day, or 28-day tumor-bearing mice were adoptively transferred to the mice 1 day after CT 26 tumor (1 x 10^5 cells) inoculation. Mice without adoptive transfer of additional CD8^+ T cells were used as controls. Data represent mean ± SEM of n = 5 mice. Results are representative of three experiments. *, p < 0.05 for statistical analysis with Mann-Whitney U test and **, p < 0.01.
FIGURE 4. Splenic CD4\(^+\)CD25\(^+\)Foxp3\(^+\) T cells from tumor-bearing mice diminishing tumor-inhibition potency of CD8\(^+\) T cells. A, Absolute number of Treg cells in spleen, draining lymph node or non-draining lymph node of naive mice, day-7 tumor-bearing mice, and 28-day tumor-bearing mice. Data represent mean ± SEM from \(n = 6\) mice in each group. B, Splenic CD8\(^+\), CD4\(^+\)CT25\(^+\), and CD4\(^+\)CD25\(^+\) T cells were purified from day-28 tumor-bearing mice. CD8\(^+\) T cells alone (group A), or together with CD4\(^+\)CT25\(^+\) (group B) or CD4\(^+\)CD25\(^+\) T cells (group C) at a ratio of 1:0.5, were cocultured with irradiated CT 26 cells (1:0.01). Supernatants were harvested 48 h later and assayed for IFN-\(\gamma\) by ELISA. C, Tumors were inoculated on day 0. On day 1, different sorted cells from 28-day tumor-bearing mice like CD8\(^+\) T cells alone (1 \times 10^6 per mice, group A), or together with CD4\(^+\)CD25\(^+\) T cells (0.3 \times 10^6 per mice, group B) or with CD4\(^+\)CT25\(^+\) T cells (0.3 \times 10^6 per mice, group C) were adoptively transferred. Growth patterns of tumors are shown. Results are mean ± SEM for \(n = 4\) mice and representative of three experiments. D, CD8\(^+\) T cells from day-28 tumor-bearing mice alone (group A) or mixed either with CD4\(^+\)CD25\(^+\) T cells from naive mice (group B) or cells from day-28 tumor-bearing mice (group C) in different ratios were then stimulated in vitro with irradiated CT 26 tumor cells for 48 h. Supernatants were harvested and assayed for IFN-\(\gamma\) by ELISA. E, Growth patterns of tumors in mice that received tumor challenge on day 0 and received CD8\(^+\) T cells alone (1 \times 10^6 per mice, group A), or together with day-28 CD4\(^+\)CD25\(^+\) T cells (0.3 \times 10^6 per mice, group B) or with naive CD4\(^+\)CD25\(^+\) T cells (0.3 \times 10^6 per mice, group C) on day 1 are shown. Mice that received tumor challenge but without any T cell transfer served as controls (group D). Results are mean ± SEM for \(n = 4\) mice and representative of two experiments. \(\ast, p < 0.05\) for statistical analysis with Mann-Whitney \(U\) test and \(\ast\ast, p < 0.01\).
day-28 CD8\(^+\) T cells to be more potent than in naïve Treg cells (Fig. 4D). The adoptive transfer studies by transferring different T cell populations into the naïve mice 1 day after primary CT 26 tumor challenge also revealed that day-28 Treg cells exhibit more potent suppression effects than those of naïve Treg cells on tumor-inhibiting ability of day-28 CD8\(^+\) T cells (Fig. 4E).
DAY-28 Treg cells would appear more potent than naïve Treg cells in terms of suppression of tumor inhibition imposed by day-28 CD8\(^+\) T cells. We examined the phenotypes of day-28 Treg cells and compared them with Treg cells from naive mice. As shown in Fig. 5A, the expression of GITR, Lag-3, and CTLA-4 was increased in day-28 Treg cells when compared with naïve Treg cells. The percentage of CD127-expressed Treg cells increased and CD62L\(^{hi}\) Treg cells decreased in day-28 Treg cells as well. In addition, expression of effector molecules like Fasl, granzyme B, perforin, and ICOS were increased in day-28 Treg cells (Fig. 5A).

A similar situation was found in Treg cells from TILs in day-28 tumor-bearing mice (Fig. 5B). Expression of Foxp3, GITR, and CTLA-4 were higher in Treg cells from TILs than in naïve Treg cells (Fig. 5B). These observations imply that Treg cells in tumor-bearing mice were more activated than in naïve mice. We analyzed the percentage of effector/memory Treg cells in tumor-bearing mice, defined as CD103\(^+\) T cells, as suggested by Huehn et al. (22). As shown in Fig. 5C, during tumor progression, the percentage of CD103\(^+\) Treg cells increased rapidly in TILs and then in spleens of tumor-bearing mice. Effector molecules and inflamed tissue-homing chemokines like CCR3 and CCR5 were highly expressed in CD103\(^+\) Treg cells when compared with CD103\(^-\) Treg cells, in both naïve and tumor-bearing mice (Fig. 5D).

**Effector/memory Treg cells are responsible for loss of tumor-inhibiting potency of day-28 CD8\(^+\) T cells**

The increased effector/memory Treg cell counts in tumor-bearing mice imply that these cells might be responsible for the loss of concomitant tumor immunity. We sorted CD103\(^+\) Treg cells and CD103\(^-\) Treg cells from day-28 splenocytes (Fig. 6A). These cells were cotransferred with day-28 CD8\(^+\) T cells into naive mice and recipient mice were subsequently challenged with CT 26 tumor cells. As shown in Fig. 6B, CD103\(^+\) Treg cells from day-28 splenocytes were more potent than CD103\(^-\) Treg cells from day-28 splenocytes in suppressing day-28 CD8\(^+\) T cell tumor-inhibition potency. We inoculated naïve mice with CT 26 tumor cells (for primary tumor, day 0) and after 6 days, we adoptively transferred day-28 CD103\(^+\) Treg cells or day-28 CD103\(^-\) Treg cells (day 6). Secondary CT 26 tumor cells were inoculated 1 day later (for secondary tumor, day 7). Results showed that CD103\(^+\) Treg cells were again more potent than CD103\(^-\) Treg cells in inhibiting the concomitant tumor immunity (Fig. 6C). Therefore, development of effector/memory Treg cells seems associated with an overall increase in Treg cell counts, attributed to inhibition of concomitant tumor immunity.

**Discussion**

Concomitant tumor immunity was described decades ago in a series of elegant studies (1). Initially, this phenomenon seemed limited to tumors with high immunogenicity (7). However, recent studies have shown that Treg cells can be induced in tumor-bearing mice, especially in tumor environments with high immunogenicity. Our current work demonstrates that Treg cells in tumor-bearing mice can be induced and that these Treg cells are responsible for the loss of tumor inhibition. This finding suggests that Treg cells play a crucial role in the development of concomitant tumor immunity.

**Day-28 Treg cell count increases in spleen and TILs of tumor-bearing mice**

Figure 6 shows the increase in Treg cell counts in spleen and TILs of tumor-bearing mice. The figure compares the Treg cell counts in day-28 spleen and TILs of tumor-bearing mice with those in naive mice. The results indicate that Treg cell counts increase significantly in both spleen and TILs of tumor-bearing mice, suggesting that Treg cells are activated and expanded in these tumor environments.
literature has shown that tumors with low immunogenicity still possess concomitant tumor immunity if Treg cells are depleted (9). Concomitant tumor immunity seems a general phenomenon involving a large variety of murine tumors with different immunogenicities; Treg cells might play a significant role in inhibiting this phenomenon. In this study, concomitant tumor immunity was gradually lost as tumors progressed (Fig. 1C). CD8+ T cells from mice that lost concomitant tumor immunity had the greatest tumor-inhibition potency (Figs. 2 and 3, D and E). Effector/memory Treg cells increased during this process (Fig. 5B). The CD4+CD103+Foxp3+ T cells had greater perforin, granzyme B, and FasL expression, whereas CCR3/CCR5 were increased and CCR7/CCL2L were decreased (Fig. 5D). These cells accumulated initially in TILs and later accumulated in the spleen. These effector/memory Treg cells seem responsible for the loss of concomitant tumor immunity as the tumor progresses (Fig. 6, B and C).

The fact that CD8+ T cells from spleens of mice lacking concomitant tumor immunity retained the most potent anti-tumor immunity is intriguing but not unexpected. CD8+ T cells from tumor-bearing hosts have already been suspected to have impaired in tumor-eradication potency (3). A lot of effort has been made to augment tumor-specific CTL functions. Strategies have included challenge with Ag-pulsed dendritic cell, in vitro transfer of expanded T cells harvested from TILs, transferring TILs with growth factors, and transfer of cytokine-activated T cells (10, 30–33). However, responses have been unsatisfactory, which indicates that mechanisms of tumor escape might not involve the initial stage of T cell priming (34). In this study, splenic CD8+ T cells from mice devoid of concomitant tumor immunity efficiently inhibited tumor growth if these cells were adoptively transferred 1 day after tumor challenge to naive mice (Fig. 3). These results are in line with the argument that the ability of a tumor to elude immune responses does not depend solely on CD8+ T cell priming inability.

In line with previous reports, CD8+ tumor-infiltrating T cells harvested from the tumor site were activated and expressed an enormous amount of perforin/granzyme B, even though they could not eradicate/inhibit tumors (Fig. 3B) (35, 36). The latter finding may be attributed to a defective microtubule-organizing center with defective lytic granule exocytosis (36). This result is also consistent with the notion that the tolerogenic tumor microenvironment is responsible for inactivation of the lytic function of TILs, though they are activated and expressed perforin/granzyme B (34).

A lot of evidence has demonstrated that Treg cells are one of the key mechanisms involved when tumors elude immune responses and are one of the main obstacles to tumor immunotherapy (17, 37–40). Our results indicate that Treg cells hinder concomitant tumor immunity, which is in line with previous reports (9). Our serial in vivo and in vitro studies demonstrate that Treg cells ameliorate tumor-bearing CD8+ T cell functions; this demonstration is most prominent in day-28 tumor-bearing mice. Interestingly, we also found CD103 to be highly expressed on the subgroup of Treg cells most potent in suppression of tumor-inhibition potency of CD8+ T cells. CD103 has been proposed as a marker of effector/memory Treg cells (22). Therefore, these effector/memory Treg cells can migrate to sites of tissue, and are unique in their capacity to mediate suppression of T cell activation for tissue protection (21). The roles of these effector/memory Treg cells in inhibition of immune responses have been demonstrated in several models, including colitis and in a graft-vs-host condition (32, 41–43). The evidence implies that these effector/memory Treg cells are responsible for damage control, which is one of the Treg cell functions proposed by Tang and Bluestone (44). In this study, these cells also seem involved in tumor protection. It is possible tumor cells themselves or the surrounding microenvironment could enhance attraction or development of these cells, which would protect the tumor from immune attack.

It has been claimed that an integrin, CD103, is highly expressed on intraepithelial lymphocytes in the gut wall and other epithelial compartments, such as the skin, liver, and lung (45, 46). However, the tissue-tropism behavior of T cells, especially Treg cells, is also associated with chemokine receptor expression that includes CCR4 and CCR5 (11, 28, 46–49). In the present study, inflammation-related chemokine receptors such as CCR3/CCR5 were highly expressed on these CD103+ Treg cells, especially in the tumor microenvironment (Fig. 5D). This result further highlights the association of inflammation, tumors, and effector/memory Treg cell trafficking.

In addition, these effector/memory Treg cells, which we found in our tumor model, more greatly express CTLA-4/GITR/Lag-3 and perforin/granzyme B/FasL (Fig. 5A), which strongly supports the notion that these cells were activated with full effector molecules (22). The strong expression of perforin/granzyme B/FasL implies that Treg cell killing ability might be very important in the tumor microenvironment, which is in-line with a recent study that used granzyme B knockout mice in a tumor model (50).

In conclusion, this study demonstrates that CD8+ T cells from mice that have lost concomitant tumor immunity still possess potent tumor-eradicating potency. Effector/memory Treg cells with CD103 expression gradually increased as the tumor progressed. These effector/memory Treg cells were responsible for repressing tumor-inhibiting CD8+ T cells and the subsequent loss of concomitant tumor immunity.

Disclosures

The authors have no financial conflict of interest.

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