Supplementary Material and methods

Functional proliferation assay

Proliferation assays were performed as previously described (1, 2). In brief, 1 × 10^5 naïve $CD4^+$ T cells freshly purified from healthy donors were co-cultured with tumor-infiltrating γδ1 T cell lines (BTILs) or γδ2 T cells (control) at different ratios in anti-CD3-coated (2 µg/ml) 96-well plates in T cell assay medium containing 2% human AB serum. After 56 hours of culture, [^{3}H]-thymidine was added at a final concentration of 1 µCi/well, followed by an additional 16 hours of culture. The incorporation of [^{3}H]-thymidine was measured with a liquid scintillation counter.

Immunohistochemical staining and visual scoring of IP-10 expression

The γδ T cells and IP-10^+ tumor cells in cancer and normal tissues were determined using immunohistochemical staining, as we described previously (3). The frozen sections were stained with a mouse anti-human γδ TCR (clone B1.1, eBioscience) monoclonal and rabbit anti-human IP-10 (R & D Systems) antibodies, and then followed the procedure of the Histostain®-Plus 3rd Gen IHC Detection Kit (Invitrogen, CA). Controls were performed by incubating slides with the isotype control antibody instead of primary antibodies, or second antibody alone. The positive cells in tissues were evaluated manually using a computerized image system composed of a Leica ICC50 camera system equipped on a Leica DM750 microscope (North Central Instruments, Minneapolis, MN). IP-10 expression in tumor tissues were examined and scored: 0: no staining; +: low intensity of positive tumor cell staining, or <25% of of tumor cells are IP-10 positive cells; ++: medium level intensity of positive tumor cell staining, or >25% and <50% of tumor cells are IP-10 positive cells; +++: high level intensity of positive tumor cell staining, or >50% and <75% of tumor cells are IP-10 positive cells; or ++++: extremely high level intensity of positive tumor cell staining , or >75% of tumor cells are IP-10 positive cells.

***In vivo imaging* studies**

586mel-bearing Rag1$^{-/-}$ mouse model: Human 586mel tumor cells cells (5 × 10^6) in 100 μl of buffered saline were subcutaneously injected into Rag1$^{-/-}$ mice. TIL586 CD8$^+$ T cells were incubated with 320 μg/ml of XenoLight DiR (Caliper Life Science) for 30 minutes. Stained T cells were washed twice in PBS and then injected tail intravenously (5 × 10^6/mouse in 200 μl of buffered saline) into 586mel-bearing (tumor size about 10 × 10 mm) Rag1$^{-/-}$ mice. Tumor-bearing mice were injected with a neutralizing antibody against human IP-10 (clone 33036, R & D Systems) or isotype control in tumor sites at 2 hours before TIL586 cell injection, and at day 2 after T cell injection. Mice were imaged with an IVIS (Caliper Life Science) at 3 days post injection. The appropriate filter set for DiR imaging 710 nm excitation and 760 nm emission was used. The signal intensity in tumor sites was quantified in terms of photons/s/cm2/sr using Living Image 3.1 Software (Xenogen Corporation, MA).

MDA-MB-453 and mel586 tumor-bearing NSG mouse model: Mixtures of human 586mel tumor cells (4 × 10^6/ mouse) and breast MDA-MB-453 cancer cells (3 × 10^6/mouse) were subcutaneously injected into NSG mice. DiR-stained CD8$^+$ TIL586 cells (5 × 10^6/ mouse) combined with or without breast cancer-derived γδ Treg cells (3 × 10^6/mouse) were adoptively transferred through intravenous injection on day 3. Concurrently, some tumor-bearing mice were injected with either human IP-10 neutralizing antibody or isotype control antibody (40 μg/mouse) into tumor sites at 2 hours before T cell adoptive transfer and then every 5 days after their transfer. The change of signal density was checked on day 3 and 8 after TIL586 cell injection, using an IVIS.

References

1. Peng G, Guo Z, Kiniwa Y, Voo KS, Peng W, Fu T, Wang DY, Li Y, Wang HY, Wang RF. 2005. Toll-like receptor 8-mediated reversal of CD4+ regulatory T cell function. *Science* 309: 1380-4

2. Peng G, Wang HY, Peng W, Kiniwa Y, Seo KH, Wang RF. 2007. Tumor-infiltrating gammadelta T cells suppress T and dendritic cell function via mechanisms controlled by a unique toll-like receptor signaling pathway. *Immunity* 27: 334-48

3. Ma C, Zhang Q, Ye J, Wang F, Zhang Y, Wevers E, Schwartz T, Hunborg P, Varvares MA, Hoft DF, Hsueh EC, Peng G. 2012. Tumor-Infiltrating gammadelta T Lymphocytes Predict Clinical Outcome in Human Breast Cancer. *J Immunol* 189: 5029-36

Supplementary Figure legends

Supplementary Figure 1

IP-10 is the only functional chemokine inducing the migration of γδ Treg cells.

(A) Human breast cancer-derived γδ1 T cells have strong suppressive activity on the proliferation of responding $CD4^+$ T cells. γδ2 T cells isolated from healthy donors were included as a control and didn't have suppressive activity. BTILs: breast cancer-derived γδ1 T cell lines. γδ1 T cells were purified from bulk TILs by FACS and used to test their ability to inhibit responding naïve T cell proliferation. The proliferation of naïve T cells was determined by a [^{3}H]-thymidine incorporation assay. **(B)** Recombinant IP-10 has chemotactic activity for γδ Treg cells, but not $CD4^+CD25^+$ Treg cells. Various concentrations of recombinant IP-10 as indicated were added to the lower chamber instead of culture supernatants, and chemotactic activity for γδ Treg cell lines were determined. $CD4^+CD25^+$ Treg cells served as a control. Results are representative of three independent experiments with similar results.

Supplementary Figure 2

Correlation analyses of tumor-infiltrating γδ T cells and tumor IP-10 expression levels in human breast cancer tissues.

(A) Different expression levels of IP-10 *in situ* in breast tumor tissues were ranked as +, ++, +++, and ++++. Frozen tissue sections from normal and cancer tissues were immunohistochemically stained with anti-IP-10 antibody using the Histostain®-Plus 3rd Gen IHC Detection Kit. IP-10 expression levels in tumor tissues were scored. IP-10 expression in normal breast tissues was served as a control and ranked as 0. The photomicrographs are shown at ×100 and ×400 magnifications. **(B)** No significant correlation was found between numbers of γδ Treg cells infiltration and IP-10 expression levels in human breast tumor tissues. Frozen breast cancer tissue sections were immunohistochemically stained with anti-TCRγδ and anti-IP-10 antibodies, as described in (A). Tumor tissues were divided into 3 groups based on IP-10 expression levels, and then determined the numbers of γδ T cells within tissues. Bars show the average of numbers of γδ T cells.

Supplementary Figure 3

Neutralization of IP-10 does not affect the trafficking and distribution of tumor-specific effector $CD8^+$ TIL586 cells into tumor sites in tumor-bearing mice.

(A) Neutralizing antibody against human IP-10 didn't inhibit the TIL586 cell migration and trafficking into tumor tissues in 586mel-bearing $Rag1^{-/-}$ mice. XenoLight DiR stained TIL586 $CD8^+$ T cells were adoptively transferred through intravenous injection into 586mel-bearing $Rag1^{-/-}$ mice. Concurrently, tumor-bearing mice were injected with either human IP-10 neutralizing antibody or isotype control antibody (40 μg/mouse) into tumor sites at 2 hours before and then at 2 days after TIL586 cell adoptive transfer. Mice were then imaged with IVIS spectrum at day 3 post injection. Left panels are images of TIL586 in 3 tumor-bearing mice with different antibody treatments. The circles indicated the tumor growth sites. Color bars represent signal intensity scale over whole body. Right panel shows the signal intensity in tumor sites of two groups quantified in terms of photons/s/cm2/sr. Significance was determined by paired t test. **(B)** Neutralization of IP-10 didn't affect distribution of TIL586 cells in MDA-MB-453 and mel586 tumor-bearing NSG mice. Mixtures of human 586mel tumor cells (4×10^6/ mouse) and breast MDA-MB-453 cancer cells (3×10^6 / mouse) were subcutaneously injected into NSG mice. DiR-stained 586mel-specific $CD8^+$ TIL586 cells (5×10^6/ mouse) combined with or without breast cancer-derived γδ Treg cells (3×10^6/mouse) were adoptively transferred through intravenous injection on day 3. Concurrently, some tumor-bearing mice were injected with either human IP-10 neutralizing antibody or isotype control antibody (40 μg/mouse) into tumor sites at 2 hours before T cell adoptive transfer and then every 5 days after their transfer. The change of signal density was checked on day 3 and 8 after TIL586 cell injection. Color bars represent signal intensity scale over whole body.