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Peritoneal T Lymphocyte Regulation by Macrophages

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Abstract

The T cell composition of the peritoneal cavity (PerC) in naïve BALB/c, C57BL/6, DBA/2J, and B-1 B cell-defective BALB.*xid* mice was investigated. The BALB.*xid* PerC T cell pool had a high CD4:CD8 T cell ratio relative to the other strains whose ratios were similar to those found in their lymph node and spleen. All mice had significant representation of T cells with an activated (CD25⁺, GITR^{hi}, CD44^{hi}, CD45RB^{lo}, CD62L^{lo}) phenotype and low numbers of Foxp3⁺ T_{reg} cells in their PerC. Despite a phenotype indicative of activation, peritoneal T cell responses to CD3 ligation were very low for C57BL/6 and BALB.*xid*, but not BALB/c, mice. Enzyme inhibition and cytokine neutralization studies revealed active suppression of the T cell response mediated by the macrophages that represent a significant portion of PerC leucocytes. Driven by IFN γ to express iNOS, macrophages suppressed T cell activation *in vitro* by arginine catabolism. Although BALB/c T cells were also in a macrophage-dense environment their limited IFN γ production failed to trigger suppression. This difference between BALB/c and BALB.*xid* PerC T cells suggests a role for *xid* in shaping macrophage-mediated immune regulation.

Keywords

Peritoneal; T cells; macrophages; suppression

Introduction

Among numerous research applications, the murine peritoneal cavity (PerC) has served immunology as a site to study inflammation *in vivo* and as a source of activated (thioglycollate- or peptone-elicited) myeloid cells for *in vitro* studies (Fortier 2001; Davies and Gordon 2005; Turchyn et al. 2007; Boldizar et al. 2009). The resident myeloid cells of the PerC have been described both as inflammatory monocytes and as immature macrophages (LaGasse and Weissman 1996; Geissman et al. 2003; Kamei and Carman 2010). They have an increased representation in the PerC relative to the presence of the myeloid cells found in organized lymphoid tissue such as the spleen (SP) or lymph node (LN) (Turchyn et al. 2007). The PerC also contains lymphocytes, most notably the B-1 B cell subset responsible for spontaneous (“natural”) antibody production, humoral

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autoimmunity, and B-cell lymphocytic leukemia (Hardy 2006). In contrast to the wealth of knowledge regarding PerC B cell biology surprisingly little has been reported on the T cells found in this anatomic location.

In our studies of the ability of B cell subsets to present superantigens to T cells PerC cells were found to be effective APCs *in vivo* but not *in vitro* (Riggs et al. 2004; Rosini et al. 2004). Considering that PerC cells are enriched for the B cells and macrophages that serve as APCs, this result was unexpected. Subsequent research revealed that PerC macrophages, triggered by IFN γ , actively suppressed the T cell response (Matlack et al. 2006). While characterizing this observation our studies of PerC T cell biology were initiated. This report summarizes findings from this research and reveals differences in PerC T cell biology among common strains of mice. Surprisingly, rather than following their BALB/c strain of origin, BALB.*xid* PerC T cells most closely resembled those of C57BL/6 mice. These data reveal how cellular composition and cytokine milieu combine to promote macrophage regulation of lymphocyte activation. The results are relevant to advancing knowledge of the aberrant immune regulation found in tumor microenvironments.

Materials and methods

Mice

Male and female BALB.*xid* (XID), BALB/c, DBA/2J, and C57BL/6J mice, bred and maintained at Rider University, were studied under the age of 8 months. All mice were handled in accord with NIH, Animal Welfare Act, and Rider University IACUC guidelines.

Preparation of cell suspensions

LN and SP cell suspensions were obtained by gentle disruption of the organ between the frosted ends of sterile glass slides. RBCs were removed from SP cell preparations by hypertonic lysis and subsequent Ficoll-Hypaque density gradient centrifugation followed by extensive washing with HBSS (Life Technologies Inc., Grand Island, NY). PerC cells were obtained by flushing the peritoneum with 10 mls of warm (37° C) HBSS supplemented with 3% FBS (Hyclone, Logan, UT). RBCs were depleted by treatment by hypertonic lysis. Viable cell counts were determined by Trypan blue exclusion.

Cell Culture

Serial two-fold titrations ($4.0 - 0.25$ or $3.0 - 0.375 \times 10^6/\text{ml}$) of PerC Cells, in RPMI 1640 culture media (Life Technologies, Grand Island, NY) supplemented with 10% FCS (Hyclone), 0.1 mM nonessential amino acids, 100 U/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, 50 $\mu\text{g}/\text{ml}$ gentamicin, 2 mM L-glutamine, antibiotics, 2×10^{-5} M 2-ME, and 10mM HEPES, were incubated in a humidified atmosphere of 5% CO $_2$ at 37°C in 96 well microtiter plates (Costar 3894, Cambridge, MA). Anti-CD3 $_e$ (145-2C11 MAb, eBioscience, San Diego, CA) was added at 1 $\mu\text{g}/\text{ml}$ at the start of culture. All enzyme inhibitors were added as described (Matlack et al. 2006). For IFN γ SPOTELISA, cells were removed after overnight culture, washed extensively in sterile PBS, and then cultured and developed in ELISPOT plates as described by the supplier (eBioscience). For proliferation assays, after 42 hrs, 1 μCi of [^3H] thymidine was added to each well. The plates were frozen 4 hrs after labeling, then thawed for analysis using a semi-automated cell harvester (Skatron Instruments, Richmond, VA). Radioactivity was measured by scintillation spectrometry. In each experiment, 3-5 microwells were established for each test group.

Immunofluorescence staining and flow cytometric analyses

PerC cell suspensions were first blocked with rat anti-mouse CD16/32 (Fc Block, eBioscience) and 2% normal rat serum (Jackson ImmunoResearch, West Grove, PA). The

cell suspensions were then stained using titrated amounts of FITC-, Cy-Chrome-, or PE-labeled rat anti-mouse CD4, CD8, CD11b, CD25, CD44, CD45Rb, CD62L, F4/80, and/or GITR MAbs (eBioscience). Foxp3⁺ T_{regs} were stained following the supplier's protocol (eBioscience). Isotype- and fluorochrome-matched, nonspecific MAb controls were employed to establish analysis gates. The percentage of lymphocytes or myeloid cells co-expressing sets of these markers were determined via multiparameter flow cytometric analyses on a FACSCalibur™ flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA) by FSC/SSC gating of the lymphoid or myeloid population using CellQuest software.

Statistical analyses

Data sets were compared using the student's t-Test.

Results

CD4/CD8 T lymphocyte composition of the peritoneal cavity

While there has been extensive study of the B cell composition of cells collected by peritoneal lavage (Hardy 2006), there has been much less investigation of the T cell biology of this body site. We investigated the T cell composition of this anatomic location by comprehensive flow cytometric analyses. Three widely researched murine strains, BALB/c, DBA/2J, and C57BL/6J were studied as well as the B cell-defective BALB.*xid* strain. The composition of conventional lymphoid tissue (spleen {SP} and lymph node {LN}) was included for comparison. The results show significant differences in the number of CD4 and CD8 T cells found in the PerC and SP of these mice (Figure 1). Although BALB/c and DBA/2J had greater percentages of CD4 and CD8 T cells, C57BL/6 mice had the greatest number of both CD4 and CD8 T cells in their PerC (Figures 1A and B). BALB.*xid* mice had the second greatest number of PerC CD4 T cells and the lowest number of CD8 T cells. DBA/2J and BALB/c mice had similar numbers of both subsets with BALB/c having more CD4 T cells in the PerC. In the spleen, DBA/2J mice had the fewest of each T cell subset, C57BL/6 had the greatest number of CD8 T cells, and BALB/c had the greatest number of CD4 T cells (Figure 1C). T cell subset composition differences among these strains were readily demonstrated as CD4 to CD8 T cell ratios (Figure 1D). C57BL/6 mice had the lowest CD4:CD8 ratios for their SP and LN T cells. B cell defective BALB.*xid* mice had a very high CD4:CD8 ratio for their PerC T cell pool. These data illustrate that the peritoneum harbors significant numbers of CD4 and CD8 T cells.

Naïve and memory/effector T cell subsets in the peritoneal cavity

Organized lymphoid tissue has evolved to filter blood and lymph trapping antigens for the initiation of immunity. In unimmunized animals, the great majority of lymphocytes in these tissues are naïve as they have not yet encountered antigen. To assess the naïve and memory/effector composition of the PerC T cell pool in naïve mice, expression of CD44, CD62L, and CD45Rb was assessed. The results show that, relative to SP and LN, the CD4 T cells in the PerC were comprised of a significantly greater percentage of memory/effector T cells (Figure 2). Cells that were CD44^{hi}, CD62L^{lo}, and CD45RB^{lo} were the predominant CD4 T cell subset in the PerC, followed by SP, and then LN. In contrast, the predominant CD4 T cell subset in the SP and LN had the reciprocal (naïve: CD44^{lo}CD62L^{hi}CD45RB^{hi}) phenotype. CD8 T cells in the PerC were also enriched for CD44^{hi} cells (Figure 2E). These results reveal that the PerC T cell pool in unimmunized animals is enriched for activated CD4 and CD8 T cells.

GITR⁺CD25⁺ T cells are enriched in the peritoneal cavity

As a further assessment of their activation status PerC, SP, and LN T cells were compared for GITR and CD25 (IL-2R α) expression. These antigens are characteristic of activated T cells and have been used to define regulatory T cells (T_{regs}) (Gavin and Rudensky 2003). Again the CD4 T cells found in the PerC had the greatest percentage of cells expressing these antigens (Figures 3A-C). For all strains there were more GITR⁺ cells than CD25⁺ cells. The percentage of GITR-expressing cells in the CD8 T cell pool was lower than that for CD4 T cells (Figure 3D). In contrast to CD4 T cells, there were more CD25⁺ CD8 T cells than GITR⁺ CD8 T cells (Figure 3E). These results reinforce that, relative to organized lymphoid tissue, the PerC contains a greater fraction of activated T cells and reveal differences in the representation of these cells within the CD4 and CD8 subsets.

Foxp3⁺ T cells are not enriched in the peritoneal cavity

CD4 T cells that express CD25 and GITR include the regulatory T cell subset (T_{regs}). However, the best marker for T_{regs} is the transcription factor Foxp3 (Fontenot and Rudensky, 2005). Foxp3⁺ cells were a greater percentage of the total CD4⁺ T cell pool in the LN relative to the PerC in all strains except DBA/2J (Figure 4A,B). The actual number of Foxp3⁺ T cells was similar for the LN and the PerC of each strain (Fig 4C). Validation of these observations came with the analysis of C57BL/6 Foxp3 GFP knockin mice which had similar percentages and numbers of Foxp3⁺ T cells in the same tissues (data not shown). These results illustrate that, relative to conventional lymphoid tissue, the PerC is enriched for activated T cells and has a lower representation of Foxp3⁺ T_{regs} in the CD4 T cell pool.

Limited PerC T cell response unless cultured at low density

To compare their function, PerC T cells from the various strains were stimulated by CD3 ligation. Since BALB/c and DBA/2J mice had the greatest percentages of total T cells in their PerC (Figure 1.A) their T cell proliferative response was expected to be the greatest. BALB/c mice did have the greatest response at three of the four cell concentrations cultured; T cell proliferation for the other strains was markedly lower (Figure 5). The T cell response of BALB.xid and C57BL/6J mice increased as the plating concentration decreased. Although exhibiting the lowest maximal proliferative response, only the DBA/2J PerC T cell response titrated with decreasing cell density. Each strain had a distinct titration profile for the PerC T cell response to CD3 ligation, however the BALB.xid response more closely paralleled that of C57BL/6 rather than that of the BALB/c strain.

Myeloid composition of the peritoneal cavity

Increased cell-cell contact or synapse formation, particularly between lymphocytes and myeloid cells, has been implicated in immune regulation (Silberman et al. 2010). The recovery of the T cell response concurrent with the dilution of cultured PerC cells suggested that high cell density was triggering suppression. To compare the relative lymphoid/myeloid composition of PerC cells to conventional lymphoid tissue flow cytometry was conducted. Myeloid (primarily CD11b^{hi}, F4/80⁺, CD16/32⁺ macrophages) and lymphoid (primarily CD3⁺ T cells and IgM⁺, CD45R⁺ [B220] B cells) FSC/SSC gates were established based on established criteria (Figure 6, Panel A; Turlyn et al, 2007). For all strains, SP and LN had similar, low (< 5%) myeloid representation that contrasted with the greater percentage of these cells recovered from the PerC (Fig 6, Panels B, C, & D). The number of total macrophages was similar when comparing SP and PerC within individual mice but differed among the strains with C57BL/6 and BALB/c mice having more of these cells than DBA/2J and BALB.xid (Fig 6, Panel E). When these data were presented as a ratio of macrophages to T cells, the PerC had the highest ratio being 10-fold greater than that seen for SP which was 2-fold greater than LN (Fig 6, Panel F). Combined with the PerC T cell activation

results (Fig 5), these data suggested that macrophage density could impact the T cell response to CD3 ligation.

Blocking macrophage-mediated suppression recovers the PerC T cell response

To determine if macrophages suppress T cells under macrophage-dense culture conditions, PerC T cells were activated in the presence of inhibitors (1-methyl tryptophan [1-MT], N^G-monomethyl-L-arginine [1-MA], N^ω-hydroxy-nor-L-arginine [1-NA], indomethacin [INDO]) of the enzymes (indoleamine 2,3 dioxygenase [IDO], inducible nitric oxide synthase [iNOS], arginase [ARG], cyclooxygenase [COX]) known to regulate T cell activation (Matlack et al. 2006). Neutralizing MABs for IFN γ and IL-10 were also tested as these cytokines have been shown to regulate T cells in myeloid-dense environments (Gabrilovitch and Nagaraj 2009). The data, presented as a percentage of the control response (no enzyme inhibitor or MAB added), reinforced that the BALB.xid and C57BL/6 PerC T cell response profiles were similar (Fig 7). T cell activation in both strains was best recovered by blocking iNOS or IFN γ followed by COX inhibition. BALB/c PerC T cells were much less suppressed (Fig. 5) but also responded best to iNOS inhibition and had modest responses with all of the inhibitors tested. This modest response to all inhibitors was also seen with BALB.xid PerC cells when they were cultured at a lower concentration (Fig. 7). Likewise, modest DBA/2J PerC T cell recovery was noted with all inhibitors, the best following ARG inhibition. The number of IFN γ -secreting cells generated following PerC T cell activation correlated with the degree of suppression seen for each strain, ie., C57BL/6 > BALB.xid > BALB/c = DBA/2J (Fig 8). Combined, these results suggest a primary role for IFN γ -triggered macrophages in tempering T cell activation.

Discussion

Unlike peritoneal B cells, whose immunobiology has been extensively researched, T cells in this body cavity have not been studied. In this report the PerC T cell pool of common strains of mice was shown to include a high frequency of cells with an activated (CD25⁺, GITR^{hi}, CD44^{hi}, CD45RB^{lo}, CD62L^{lo}) phenotype. Relative to the cells found in organized lymphoid tissue such as spleen or lymph node, the PerC harbored a significantly lower frequency of Foxp3⁺ regulatory T cells. Considering this “pro-activation” phenotype it was interesting that PerC cells generally had a poor response to CD3 ligation. Dissection of this observation revealed that PerC macrophages, triggered by IFN γ production, were consuming arginine and actively suppressing the T cell response. This was particularly the case with the Th1 strain C57BL/6. In contrast, lower IFN γ production by the Th2 strain BALB/c permitted PerC T cell activation even in the presence of a high concentration of macrophages. It was surprising that the PerC T cell response of the BALB.xid congenic was more similar to that of the C57BL/6 response rather than that of the background (recipient) BALB/c response. These results reveal genetic differences in macrophage-mediated regulation of T cell activation at both the strain and single gene level.

Much of the prior research of PerC leucocytes has focused upon inflammatory infiltrates of thioglycollate-, casein-, or peptone-elicited macrophages and neutrophils (peritoneal exudate cells, PECs) rather than the normal “resident” population of PerC cells (Fortier 2001; Davies and Gordon 2005; Turchyn et al. 2007). Although the cellular composition varies depending upon the time post-challenge, PEC preparations are primarily comprised of myeloid cells (Lagasse and Weissman 1996; Turchyn et al. 2007). In contrast, lymphocytes, particularly B cells, outnumber myeloid cells in the PerC leucocyte pool of naïve animals (Turchyn et al. 2007). However, the peritoneum differs from organized lymphoid tissue by having greater numbers of B-1 B cells, fewer T cells, and more macrophages (Hardy 2006; Turchyn et al. 2007), factors likely to impact T cell biology both *in vivo* and *in vitro*. The strain differences in PerC T cell biology described in this report parallel differences in B-1 B cell and

macrophage composition reported among mouse strains (Stadler and de Week 1980; Hayakawa et al. 1984; Festing et al. 1990; Turchyn et al. 2007). The reduced number of B-1B cells and increased macrophage representation in XID mice likely combine to contribute to their distinctive PerC T cell biology.

How is it that BALB.xid PerC T cells are more similar to those of C57BL/6 rather than BALB/c mice? Their lower number of IL-10-producing B-1 B cells has been the most common explanation for the increased IFN γ production (Th1 phenotype) seen with XID T cells (Hoerauf et al. 1994). However, C57BL/6 mice have B-1 B cells in their PerC and they have more IFN γ -secreting cells than BALB.xid mice (Hardy 2006; Figure 8). Based on MAb neutralization studies, *in vitro* IL-10 production was not a significant factor in the T cell suppression observed in this study (Figure 7). It is more likely that the absence of B-1 B cells shaped the development (intrinsic biology) of the XID PerC T cells and macrophages (Popi et al. 2004) fostering a pro-inflammatory (Th1) phenotype that was exacerbated by culture. Reduced numbers of B cells would permit more T cell-macrophage contact *in vitro*, increasing T cell activation and triggering subsequent regulatory mechanisms. T cell activation was recovered with PerC cell dilution indicating that the number of contacts made between T cells and macrophages contributes to the T cell suppression observed *in vitro* (Figure 5; Silberman et al. 2010). In a similar fashion, high macrophage - T cell ratios have been shown to temper immunity in infectious disease (Shimizu et al. 2004, Taylor et al. 2006). There is growing interest in the myriad ways (inhibitory cytokines, prostaglandins, amino acid consumption, *etc.*) by which myeloid cells actively control the adaptive immune response particularly in relation to the immune suppression seen in certain tumors (Gabrilovitch and Nagaraj 2009).

The observation of activated T cells in the PerC of naïve mice invites the question as to what these cells are doing in this body cavity. Prior studies have shown the preferential localization of memory effector T cells to extralymphoid sites such as the PerC (Masopust et al. 2001; Reinhardt et al. 2001). This redistribution of cells following peripheral immunization provides for widespread dissemination of immunity and memory (Mackay and von Andrian 2001). However, regional immunity more likely explains the T cell biology described for the naïve mice studied here. Perhaps studies of B-1 cells are relevant as these cells traffic from blood to lymphoid aggregates (“milky spots”) within the omentum, a loose mesothelial sheet that connects the spleen, pancreas, stomach, and transverse colon (Williams and White 1986; Ansel et al. 2002). The omentum filters the lymphatic fluid that bathes the abdominal viscera providing a site for the genesis and trafficking of effectors of innate and adaptive immunity. Study of this tissue historically has focused upon “natural” antibody production by B-1 B cells (Solvason et al. 1991). However, recent work has shown that the omentum can support T cell activation (Carlow et al. 2009; Rangel-Moreno et al. 2009). Considering the dynamic flux and antigenic burden of the gut microflora, immune regulation in this environment is of paramount importance for the host. In this regard, the frequency of GITR⁺ T cells was particularly high consistent with the recently described role of this receptor in maintaining a balance of regulatory and effector T cells (van Olfen et al. 2009). The TCR specificity of PerC T cell subsets in healthy, naïve mice should be of particular interest considering that perturbation of gut microflora has been shown to promote autoimmune disease (Turley et al. 2006; Vaarala et al. 2008). Indicative of their role in controlling inflammation, PerC T_{regs} and regulatory APC expand markedly following parasite challenge (Atochina et al. 2001; McSorley et al. 2008; Walsh et al. 2009). The presence of T_{regs}, B-1 B cells, and macrophages with regulatory properties in the PerC of naïve mice reveals that immune homeostasis in this location is essential for the host.

In summary, the murine PerC was shown to house a significant number of activated T cells that, in culture, were restrained by macrophages. This regulation differed among mouse

strains indicating the utility of further investigation of PerC cell biology in gene-modified mice. Such research will serve in advancing knowledge for approaches to reactivate the dormant immunity characteristic of tumors.

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Abbreviations

LN	lymph node
PEC	peritoneal exudate cell
PerC	peritoneal cavity
SP	spleen
XID	X chromosome-linked immune-defective

References

- Ansel K, Harris R, Cyster J. CXCL13 is required for B1 cell homing, natural antibody production, and body cavity immunity. *Immunity* 2002;16:67–76. [PubMed: 11825566]
- Atochina O, Daly-Engel T, Piskorska D, McGuire E, Harn D. A schistosome-expressed immunomodulatory glycoconjugate expands peritoneal Gr1⁺ macrophages that suppress naïve CD4⁺ T cell proliferation via an IFN- γ and nitric oxide-dependent mechanism. *J. Immunol* 2001;167:4293–4302. [PubMed: 11591752]
- Boldizsar F, Tarjanyi O, Nemeth P, Mikecz K, Giant T. Th1/Th17 polarization and acquisition of an arthritogenic phenotype in arthritis-susceptible BALB/c, but not in MHC-matched, arthritis-resistant DBA/2 mice. *Int. Immunol* 2009;21:511–522. [PubMed: 19254958]
- Carlow D, Gold M, Ziltener H. Lymphocytes in the peritoneum home to the omentum and are activated by resident dendritic cells. *J. Immunol* 2009;183:1155–1165. [PubMed: 19553538]
- Davies J, Gordon S. Isolation and culture of murine macrophages. *Meth. Mol. Biol* 2005;290:91–103.
- Festing M, Legg R, Eydmann T, Brammall A. Mouse strain differences in resident peritoneal cells: a flow cytometric analysis. *Lab. Animals* 1990;24:53–62.
- Fontenot J, Rudensky A. A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3. *Nat. Immunol* 2005;6:331–337. [PubMed: 15785758]
- Fortier A. Activation of murine macrophages. *Curr. Prot. Immunol.* May;2001 Chapter 14; Unit 14.4.
- Gabrilovitch D, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat. Rev. Immunol* 2009;9:162–174. [PubMed: 19197294]
- Gavin M, Rudensky A. Control of immune homeostasis by naturally arising regulatory CD4⁺ T cells. *Curr. Opin. Immunol* 2003;15:690–696. [PubMed: 14630204]
- Geissman F, Jung S, Littman D. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* 2003;19:71–82. [PubMed: 12871640]
- Hardy R. B-1 B cells: development, selection, natural autoantibody and leukemia. *Curr. Opin. Immunol* 2006;18:547–555. [PubMed: 16879952]
- Hayakawa K, Hardy R, Honda M, Herzenberg LA, Steinberg A. Ly-1 B cells: functionally distinct lymphocytes that secrete IgM autoantibodies. *Proc. Natl. Acad. Sci. USA* 1984;81:2494–2498. [PubMed: 6609363]
- Hoerauf A, Solbach W, Lohoff M, Rollinghoff M. The Xid defect determines an improved clinical course of murine leishmaniasis in susceptible mice. *Int. Immunol* 1994;6:1117–1127. [PubMed: 7981141]

- Kamei M, Carman C. New observations on the trafficking and diapedesis of monocytes. *Curr. Opin. Hematol* 2010;17:43–52. [PubMed: 19996887]
- LaGasse E, Weissman I. Flow cytometric identification of murine neutrophils and monocytes. *J. Immunol. Meth* 1996;197:139–150.
- Mackay C, von Andrian U. Memory T cells- local heroes in the struggle for immunity. *Science* 2001;291:2323–2324. [PubMed: 11269302]
- Masopust D, Vezys V, Marzo A, Lefrancois L. Preferential localization of effector memory cells in nonlymphoid tissue. *Science* 2001;291:2413–2417. [PubMed: 11264538]
- Matlack R, Yeh K, Rosini L, Gonzalez D, Taylor J, Silberman D, Pennello A, Riggs J. Peritoneal macrophages suppress T cell activation by amino acid catabolism. *Immunology* 2006;117:386–395. [PubMed: 16476058]
- McSorley H, Hargus Y, Murray J, Taylor M, Maizels R. Expansion of Foxp3⁺ regulatory T cells in mice infected with the filarial parasite *Brugia malayi*. *J. Immunol* 2008;181:6456–6466. [PubMed: 18941236]
- Popi A, Lopes J, Mariano M. Interleukin-10 secreted by B-1 cells modulates the phagocytic activity of murine macrophages in vitro. Macrophages from wild-type mice have a lower ability to release nitric oxide and hydrogen peroxide when compared with macrophages from Xid mice. *Immunol* 2004;113:348–358.
- Rangel-Moreno J, Moyron-Quiroz J, Carragher D, Kusser K, Hartson L, Moquin A, Randall T. Omental milky spots develop in the absence of lymphoid tissue-inducer cells and support B and T cell responses to peritoneal antigens. *Immunity* 2009;30:731–740. [PubMed: 19427241]
- Reinhardt R, Khoruts A, Merica R, Zell T, Jenkins M. Visualizing the generation of memory CD4 T cells in the whole body. *Nature* 2001;410:101–105. [PubMed: 11242050]
- Riggs J, Howell K, Taylor J, Mahjied T, Prokopenko N, Alvarez J, Coleman C. Mls presentation by peritoneal cavity B cells. *Immunobiol* 2004;209:255–264.
- Rosini L, Matlack R, Taylor J, Howell K, Yeh K, Pennello A, Riggs J. Nonlymphoid peritoneal cells suppress the T cell response to Mls. *Immunobiol* 2004;209:575–584.
- Shimizu T, Sano C, Tomioka H. The role of B7 molecules in the cell contact-mediated suppression of T cell mitogenesis by immunosuppressive macrophages induced with mycobacterial infection. *Clin. Exp. Immunol* 2004;135:373–379. [PubMed: 15008968]
- Silberman D, Bucknum A, Kozlowski M, Matlack R, Riggs J. Cytokine treatment of macrophage suppression of T cell activation. *Immunobiol* 2010;215:70–80.
- Solvason N, Lehuenm A, Kearney J. An embryonic source of Ly1 but not conventional B cells. *Int. Immunol* 1991;3:543–550. [PubMed: 1679664]
- Stadler B, de Week A. Flow cytometric analysis of mouse peritoneal macrophages. *Cell. Immunol* 1980;54:36–48. [PubMed: 6157492]
- Taylor M, Harris A, Nair M, Maizels R, Allen J. F4/80⁺ alternatively activated macrophages control CD4⁺ T cell hyporesponsiveness at sites peripheral to filarial infection. *J. Immunol* 2006;176:6918–6927. [PubMed: 16709852]
- Turchyn L, Baginski T, Renkiewicz R, Lesch C, Mobley J. Phenotypic and functional analysis of murine resident and induced peritoneal macrophages. *Comp. Med* 2007;57:574–580. [PubMed: 18246870]
- Turley S, Lee J-W, Dutton-Swain N, Mathis D, Benoist C. Endocrine self and gut non-self intersect in the pancreatic lymph nodes. *Proc. Natl. Acad. Sci* 2005;102:17729–17733. [PubMed: 16317068]
- Vaarala O, Atkinson M, Neu J. The “perfect storm” for type 1 diabetes: the complex interplay between intestinal microbiota, gut permeability, and mucosal immunity. *Diabetes* 2008;57:2555–2562. [PubMed: 18820210]
- van Olfen R, Koning N, van Gisbergen K, Wensveen F, Hoek R, Boon L, Hamann J, van Lier R, Nolte M. GITR triggering induces expansion of both effector and regulatory CD4⁺ T cells in vivo. *J. Immunol* 2009;182:7490–7500. [PubMed: 19494272]
- Walsh K, Brady M, Finlay C, Boon L, Mills K. Infection with a helminth parasite attenuates autoimmunity through TGF- β -mediated suppression of Th17 and Th1 responses. *J. Immunol* 2009;183:1577–1586. [PubMed: 19587018]

Williams R, White H. The greater omentum: its applicability to cancer surgery and cancer therapy. *Curr. Probl. Surg* 1986;23:789–865. [PubMed: 3780294]

Fig. 1.

CD4/CD8 T cell composition of the peritoneal cavity compared with organized lymphoid tissue. PerC, SP, and LN cells of the mouse strains listed were stained for CD4 and CD8 expression as described in Methods. Data are presented as the average ($n = 7-77$) $\pm$ SEM (Panels A, B, C) or as a ratio of the number of CD4 to CD8 T cells (Panel D).

Fig. 2.

Naïve/memory T cell subset composition of the peritoneal cavity. PerC, SP, and LN cells were stained for coexpression of CD4 and CD44 (Panel B), CD62L (Panel C), or CD45Rb (Panel D) or coexpression of CD8 and CD44 (Panel E). Representative FACS plots for C57BL/6J cells are shown in Panel A. Bold numbers indicate the percentage of CD4 T cells that are CD44^{hi}, CD62L^{lo}, or CD45Rb^{lo}. The data in Panels B-E are presented as the average ($n = 6-65$) $\pm$ SEM percentage of CD4⁺ (Panels B, C, D) or CD8⁺ T cells (Panel E) expressing the phenotype listed.

Fig. 3.

GITR⁺CD25⁺ T cells are enriched in the peritoneal cavity. PerC, SP, and LN cells were stained for coexpression of CD4 and GITR (Panel B) or CD25 (Panel C) or coexpression of CD8 and GITR (Panel D) or CD25 (Panel E). Representative FACS plots for C57BL/6J cells are shown in Panel A. Bold numbers indicate the percentage of CD4 T cells that are GITR⁺ or CD25⁺. The data in Panels B-E are presented as the average ($n = 7-21$) $\pm$ SEM percentage of CD4⁺ (Panels B, C) or CD8⁺ T cells (Panels D, E) expressing the phenotype listed.

Fig. 4. Foxp3⁺ regulatory T cells are not enriched in the peritoneal cavity. PerC and LN cells were stained for coexpression of CD4 and Foxp3 as described in Methods. Representative FACS plots for C57BL/6J PerC and LN cells are shown in Panel A. Bold numbers indicate the percentage of CD4 T cells that are Foxp3⁺. The data in Panels B and C are presented as the average (n = 3-6) ± SEM percentage of CD4⁺ (Panel B) or number of CD4⁺ (Panel C) T cells expressing Foxp3.

Fig. 5.

Limited peritoneal cavity T cell response unless cultured at low density. PerC cells were cultured as described in Methods. Each point represents the average ($n = 4$ wells) CPM $\pm$ SEM. The experiment presented is representative of 6 conducted.

Fig. 6.

Myeloid cell composition of the peritoneal cavity. PerC, SP, and LN cells were stained for coexpression of F4/80 and CD11b as described in Methods. Representative FACS plots for C57BL/6J PerC, SP, and LN cells are shown in Panel A. The data in Panels B-F are presented as the average percentage or number ($n = 7-12$) $\pm$ SEM of gated cells. Panel F depicts the ratio of the percentage of macrophages to T cells.

Fig. 7.

Inhibition of macrophage suppression recovers the PerC T cell response. PerC cells from the strains listed were cultured as described in Methods. Each point represents the ratio ($n = 6$ experiments) of the experimental (plus inhibitor or neutralizing MAb) to control (media alone) average CPM. Filled bars are data for PerC cells cultured at 3.0×10^6 /ml, clear bars 1.5×10^6 /ml.

Fig. 8.

IFN γ production by PerC cells. PerC cells from the strains listed were cultured as described in Methods. Each point represents the average number ($n = 9-50$ wells tested) of IFN γ -secreting cells (SC) / 10⁶ PerC cells $\pm$ SEM. * = $p < .05$, ** = $p < .005$, *** = $p < .0005$.