CD48 Controls T-Cell and Antigen-Presenting Cell Functions in Experimental Colitis

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Background & Aims: The cell-surface receptor CD48 is a lipid-anchored protein expressed on all antigen-presenting cells and T cells. CD2 and 2B4 are known ligands for CD48, which themselves are expressed on the surface of hematopoietic cells. Here we examine the effect of CD48 in the development of chronic experimental colitis and how CD48 affects adaptive and innate immune functions. *Methods:* The role of CD48 in experimental colitis was first assessed by transferring $CD4^+CD45RB^{hi}$ cells isolated from either wild-type or $CD48^{-/-}$ mice into either Rag-$2^{-/-}$ or $CD48^{-/-}$ × Rag-$2^{-/-}$ mice. Development of chronic colitis in these adoptively transferred mice was assessed by disease activity index, histology, and production of interferon-γ in mesenteric lymph nodes. Relevant functions of $CD48^{-/-}CD4^+$ T cells and $CD48^{-/-}$ macrophages were examined using in vitro assays. In a second set of experiments, the efficacy of anti-CD48 in prevention or treatment of chronic colitis was determined. *Results:* $CD48^{-/-}CD4^+$ cells induced colitis when transferred into Rag-$2^{-/-}$ mice, but not when introduced into $CD48^{-/-}$ × Rag-$2^{-/-}$ recipients. However, both recipient mouse strains developed colitis upon adoptive transfer of wild-type $CD4^+$ cells. Consistent with a $CD4^+$ T-cell defect was the observation that in vitro proliferation of $CD48^{-/-}CD4^+$ T cells was impaired upon stimulation with $CD48^{-/-}$ macrophages. In vitro evidence for a modest macrophage functional defect was apparent because $CD48^{-/-}$ macrophages produced less tumor necrosis factor α and interleukin 12 than wild-type cells upon stimulation with lipopolysaccharide. Peritoneal macrophages also showed a defect in clearance of gram-negative bacteria in vitro. Treatment of the $CD4^+CD45RB^{hi}$→Rag-$2^{-/-}$ mice or the wild-type BM→tgε26 mice with anti-CD48 (HM48-1) ameliorated development of colitis, even after its induction. *Conclusions:* Both CD48-dependent activation of macrophages and CD48-controlled activation of T cells contribute to maintaining the inflammatory response. Consequently, T cell–induced experimental colitis is ameliorated only when CD48 is absent from both T cells and antigen-presenting cells. Because anti-CD48 interferes with these processes, anti-human CD48 antibody treatment may represent a novel therapy for inflammatory bowel disease patients.

Inflammatory bowel diseases (IBD), ie, Crohn's disease and ulcerative colitis, are chronic relapsing disorders mediated by an imbalance of the immune response to bacterial and self-antigens induced by the colonic bacteria.[1] Experimental models of chronic colitis have advanced our understanding of the many pathways involved in the pathogenesis of IBD. In many of the mouse models of chronic colitis, eg, the $CD4^+CD45RB^{hi}$ transfer model and the bone marrow transplant (BM)→tgε26 model,[2–4] disease depends on a T-helper (Th)1-like response characterized by a high concentration of interferon (IFN)-γ, interleukin (IL)-12, and tumor necrosis factor (TNF)-α in the inflamed tissue and in the serum.[2–5] Colitis in this model develops because the specific response of $CD4^+$ Th1-like cells to luminal antigens.[6–8] Consequently, experimental chronic colitis can be blocked by anti–TNF-α and sLTβR.[9–11] In the healthy wild-type (*wt*) mouse, pathogenic $CD4^+CD45RB^{hi}$ T cells are inhibited by $CD4^+CD25^+$ T regulatory (Treg) cells.[12]

Optimal activation of the T cells not only requires triggering of the T cell receptor (TCR) by major histocompatibility complex/antigen, but also depends on costimulatory molecules that bind to their ligands on the antigen-presenting cells (APCs). A number of costimu-

Abbreviations used in this paper: APC, antigen-presenting cell; BM, bone marrow; DAI, Disease Activity Index; DO11.10-TCRtg, DO11.10 TCR transgenic; ELISA, enzyme-linked immunosorbent assay; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; Mφ, macrophage; MLN, mesenteric lymph node; NK, natural killer; pMφ, peritoneal macrophages; TCR, T-cell receptor; Th, T helper; TNF, tumor necrosis factor; Treg, T regulatory; *wt*, wild type.

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latory molecules and their counterparts on APCs, ie, macrophages (Mφ) and dendritic cells, have been implicated in controlling the pathogenesis of colitis.[1,13,14] CD48 and its ligands CD2 and CD244 (2B4) are such a receptor/counterstructure set; CD48 and CD2 are, in fact, expressed on the surface of both T lymphocytes and APCs.[15,16] CD48 is a glycerylphosphatidylinositol-anchored protein expressed on T, B, and natural killer (NK) cells and all types of APCs. CD2 is expressed on the surface of T cells and APCs, whereas 2B4 contributes to the activation of NK and $CD8^+$ cells.[16] Here we examine the role of CD48 in the pathogenesis of experimental colitis by making use of $CD48^{-/-}$ and $CD48^{-/-} \times$ Rag-$2^{-/-}$ mice and a monoclonal anti-CD48 antibody. The outcomes of our experiments lead to the conclusion that CD48 is critical for the APCs and T-cell interaction and, therefore, for colitis induction. In addition, and more importantly for practical considerations, treatment of chronic colitis with anti-CD48 ameliorated established colitis.

Materials and Methods

Mice

CBA, BALB/c, and DO11.10 TCR transgenic (DO11.10-TCRtg) BALB/c mice were purchased from Jackson Laboratories (Bar Harbor, ME). BALB/c × Rag-$2^{-/-}$ mice were purchased from Taconic Farms (Germantown, NY). $CD48^{-/-}$ BALB/c and DO11.10-TCRtg × $CD48^{-/-}$ BALB/c mice were kindly provided by Dr Arlene H Sharpe.[17] $CD48^{-/-}$ mice were crossed with Rag-$2^{-/-}$ mice at the Beth Israel Deaconess Medical Center Research Animal Facility to generate $CD48^{-/-} \times$ Rag-$2^{-/-}$ mice. Tgε26 mice[18] were maintained at the Beth Israel Deaconess Medical Center Research Animal Facility. The mice were kept under specific pathogen-free conditions at the Beth Israel Medical Center Research Animal Facility. Recipient and donor mice were between 8 and 12 weeks of age. Experiments were performed along the guidelines approved by Beth Israel Deaconess Medical Center's committee.

Bone Marrow and T-Cell Transfers

The $CD45RB^{hi}$ model was performed as previously described.[2,13] Briefly, $CD4^+$ splenocytes from WT or $CD48^{-/-}$ donors were labeled with phycoerythrin-CD45RB (16A) and sorted in $CD45RB^{hi}$ naive T cells and $CD45RB^{low}$ Treg. Then 2.5×10^5 $CD45RB^{hi}$ alone or in combination with 5×10^4 $CD45RB^{low}$ Treg cells were resuspended in 400 μL of phosphate-buffered saline and injected in the tail veins of Rag-$2^{-/-}$ or $CD48^{-/-} \times$ Rag-$2^{-/-}$ recipients. Tgε26 mice were irradiated with 450 rads, and 5×10^6 T-cell–depleted CBA BM cells were injected in the tail vein, as described previously.[8] $CD45RB^{hi}$→Rag-$2^{-/-}$ recipients or BM→tgε26 mice were killed when signs of diarrhea, hunching, and wasting disease became manifest.

Antibody Treatment

In antibody prevention experiments, 200 μg of anti-CD48 (HM48-1)[19] or hamster immunoglobulin (Ig)G (Jackson Immunoresearch Laboratories, Inc) were injected per mouse twice a week starting at the time of the cell transfers. $CD45RB^{hi}$→Rag-$2^{-/-}$ recipients or BM→tgε26 mice were killed when signs of diarrhea, hunching, and wasting disease became manifest. For antibody treatment experiments of $CD45RB^{hi}$→Rag-$2^{-/-}$, recipients were injected by following the pattern described previously but starting at week 3 after the transfer, when signs of colitis were apparent. Mice received antibody treatment for 3–4 weeks and were killed when signs of colitis were apparent in the control group that was injected with hamster IgG.

Cell Preparation, Stimulation, and Cytokine Analysis

Lymphocytes from the mesenteric lymph node (MLN) of transferred mice were isolated, and $5–10 \times 10^4$ cells were placed in triplicates in 96-well plates and stimulated with plate-bound anti-CD3 antibody (10 μg/mL, 145-2C11; eBioscience, Inc, San Diego, CA) for 36 hours,[14] and then supernatants were collected and frozen. For IFN-γ detection in the colon supernatant, 100 mg of colon was extensively washed and cultured in 1 mL of RPMI-1640 (GIBCO, BRL) with 10% fetal calf serum (complete media) for 36 hours. IFN-γ was detected by enzyme-linked immunosorbent assay (ELISA; OPTEIA, BD PharMingen, Inc, San Diego, CA) by following the manufacturer's instructions. The limit of IFN-γ detection was 125 pg/mL.

For the in vitro suppression assay, 1×10^5 $CD4^+CD25^-$ T cells were isolated[13] and cocultured with 1×10^5 irradiated T-cell–depleted splenocytes as APCs and soluble anti-CD3 (1 μg/mL). $CD48^{-/-}CD4^+CD25^+$ Treg cells were added in different concentrations. Proliferation after 72 hours was assessed by the uptake of H^3 thymidine added to the culture for the last 18 hours.

For in vitro stimulation experiments, 2×10^5 peritoneal thioglycolate-stimulated Mφ (pMφ) were pulsed overnight with cecal antigen from donor mice,[8] washed 3 times, irradiated (3000 rads), and incubated with 1×10^5 $CD4^+$ T cells from the spleen, which were purified by using negative selection columns (R&D Systems, Minneapolis, MN). Cultures were incubated in triplicate in 96-well plates for 3 days and pulsed with H^3 thymidine for the last 12 hours. For stimulation of $CD4^+$ T cells with APCs, 1×10^5 T-cell–depleted splenocytes were irradiated (3000 rads) and cultured with purified 5×10^4 $CD4^+$ T cells as described previously, with soluble anti-CD3 antibody (1 μg/mL). After 48 hours, proliferation was assessed as described previously.

For cytokine production of $CD4^+$ T cells, 10^6 cells per milliliter purified $CD4^+$ T cells from spleens of *wt* or $CD48^{-/-}$ mice were stimulated in vitro with plate-bound anti-CD3 (10

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