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Function of CD27 in helper T cell differentiation

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ABSTRACT

Differentiation of naïve CD4$^+$ T cells to functional effector T-helper (T_H) cells is driven by both costimulatory molecules and cytokines. Although polarizing cytokines can induce the differentiation into a particular T_H-subset, certain costimulatory molecules also seem to affect this polarization process. We have previously found that CD70-transgenic (CD70TG) mice develop large numbers of IFN-γ-producing CD4$^+$ T cells and we therefore questioned whether CD27 triggering provides an instructive signal for T_H1 differentiation or rather supports T_H cell formation in general. Although CD70TG mice on a T_H1-prone C57Bl/6J background develop more T_H1 cells, we found that this phenotype is lost when CD70TG mice are fully backcrossed on a T_H2-prone Balb/c background, but is not replaced with more T_H2 cells. Furthermore, CD70-overexpression is not sufficient to drive T_H17 cell formation, nor does it affect the generation of FoxP3$^+$ regulatory T cells. Using an *in vitro* setting, we found that CD27-triggering does not provide instructive signals for a specific T_H cell subset, but, depending on the cytokine milieu and genetic background, supports T_H1 cell formation, while it inhibits the formation of T_H17 but not T_H2 cells. Induction of allergic airway inflammation in CD70TG Balb/c mice further illustrates that CD27 plays a supportive role in T_H1 differentiation *in vivo*, without modulating the classical T_H2 response. This supportive role of CD27 in T_H cell polarization could not be attributed to a specific change of transcription factor expression levels. In summary, this study indicates that CD27 signalling does influence T_H cell differentiation, but that it is highly dependent on the conditions and genetic background.

1. Introduction

Recognition of a MHC-peptide complex via the TCR is the first signal required for effector T cell formation, as it initiates T cell activation and clonal expansion. Subsequent to MHC-peptide binding, T cells depend on a second signal for their survival and proliferation, which is provided in the form of costimulatory molecules. For the final differentiation and polarization to effector cells, activated T cells require a third signal, which is provided by polarizing cytokines (reviewed in [1,2]). Thus, naïve T cells rely on a triad of signals for their activation and differentiation into an effector population. The large number of molecules that have been implicated in this process either instruct, support or permit the formation of a specific effector T cell population.

Within the CD4 T cell population, a large variety of helper T (T_H) cell subsets has been identified, such as T_H1, T_H2, T_H17, T_H3, T_R1 and T_{Regs}, which have been attributed a specific function in the immune system. Classically, these T_H subsets can be distinguished by their cytokine production and/or transcription factor expression. As such, T_H1 cells are characterized by the ability to produce high levels of IFN-γ and TNF-α, thereby supporting cell-mediated immunity. On the other hand, humoral immunity is linked to T_H2 formation and increased secretion of IL-4, IL-5, IL-6, IL-10 and IL-13 (reviewed in [3]). Protective anti-bacterial immunity as well as development of autoimmunity is generally linked to an increase in IL-17 producing T_H17 cells [4,5]. Next to these effector T cell subsets, two inducible regulatory T cell subsets can be identified by their production of IL-10 and TGF-β, which are respectively referred to as T_R1 and T_H3 cells [6–8]. Finally, naturally occurring regulatory T cells (T_{Regs}) are not characterized by their cytokine production,

Abbreviations: AAI, allergic airway inflammation; APC, antigen presenting cell; T_H, helper T cell; TNFR, tumour necrosis factor receptor.

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but are generally distinguished by their expression of the transcription factor FoxP3 [9,10]. The function of these T_{Reg} subsets is to regulate inflammatory responses and to prevent the induction of autoimmunity.

The differentiation of naïve $CD4^+$ T cells towards these different helper T cell lineages is classically driven by polarizing cytokines, which affect the expression and/or function of instructive transcription factors. T_H1 polarization occurs subsequent to the production of IL-12 by antigen presenting cells (APCs), which results in the upregulation of the transcription factor T_{Bet} in T cells [11]. In contrast, T_H2 formation is enhanced following IL-4 signalling and through the upregulation of the transcription factor GATA-3 [12]. Commitment of a T cell to the T_H17 lineage is induced by the transcription factor RORγt. In mice, this transcription factor is upregulated by the combination of TGF-β and IL-6, whereas in humans the combination of TGF-β and IL-1β is necessary [5,13–16]. Regulatory T cells are induced by increased levels of TGF-β and retinoic acid and result in the upregulation of the transcription factor FoxP3 [17–20].

Although polarizing cytokines clearly fulfill a key function in T_H cell formation, costimulatory molecules may also play an important role in T cell differentiation and polarization. Several lines of evidence suggest that engagement of the TNFR superfamily member CD27 by its ligand CD70 enhances T_H1 cell development. Whereas CD70 is only transiently expressed on APCs and lymphocytes during immune activation [21–25], we have previously shown that constitutive expression of CD70 on B cells induces a strong increase in the numbers of IFN-γ producing $CD4^+$ and $CD8^+$ T cells, thereby enhancing T cell mediated immunity [25–28]. In addition, microarray analysis of activated effector-type $CD4^+$ T cells from WT vs CD27-deficient mice revealed that CD27-triggering can induce a Th1-like gene expression profile [29]. In human T cells, CD27 ligation drives proliferation of $CD4^+$ T cells, but also T_H1 polarization via upregulation of IL-12Rβ2 and T_{Bet} [30]. Moreover, human TNFα-induced $CD70^+$ DCs can evoke T_H1, but also T_H17 responses, although it was not shown whether these responses are indeed dependent on CD27-engagement [31]. Finally, CD27 ligation in mice can under certain conditions promote T_H1 cell formation independently of IL-12 [32]. Overall, these data suggest that CD27, as a typical "signal 2", can directly induce T_H1 cell differentiation without the need for the classical "signal 3".

Based on these observations, we questioned whether triggering through CD27 provides instructive signals for T_H1 differentiation, or that it rather supports the formation of T_H1 cells. As the genetic background of mice has been associated with a predisposition to T_H cell polarization and disease development [33–36], we decided to approach our question by backcrossing CD70TG mice from a T_H1-prone C57Bl/6J to a T_H2-prone Balb/c background. Our data indicate that the strong T_H1 skewing observed in CD70TG mice is highly dependent on the genetic background, as it does not induce T_H1, nor T_H2 skewing on a Balb/c background. Importantly, CD27 ligation during the induction of allergic airway inflammation (AAI), a typical T_H2 response, enhanced the generation of T_H1 cells without affecting the formation of T_H2 cells. Together with *in vitro* polarization studies, our data indicate that CD27 does not instruct, but rather supports the formation of T_H1 cells, both *in vitro* and *in vivo*.

2. Materials and methods

2.1. Mice

CD70TG mice were generated on a C57Bl/6J background, maintained heterozygously and bred in the animal department of the Academic Medical Center (Amsterdam, The Netherlands) under specific-pathogen-free conditions [26]. To generate CD70TG Balb/c mice, mice were backcrossed 10x with wild type (WT) Balb/c mice (Harlan). WT mice were obtained from CD70TG C57Bl/6J or Balb/c littermates. Mice were used at 6–12 weeks of age, age- and sex-matched within experiments and were handled in accordance with institutional and national guidelines.

2.2. Cell staining and flow cytometry

Single-cell suspensions were obtained by mincing the specified organs through 40 μm cell strainers (Becton Dickinson). Erythrocytes were lysed with an ammonium chloride solution and cells were subsequently counted using an automated cell counter (Casy, Schärfe system). Cells (5×10^5–5×10^6) were collected in staining buffer (PBS with 0.5% bovine serum albumin (Sigma)) and stained for 30 min at 4 °C with antibodies in the presence of anti-CD16-CD32 (FcBlock, clone 2.4G2; kind gift from Dr. Louis Boon, Bioceros, The Netherlands). The following monoclonal antibodies were obtained from Pharmingen: allophycocyanin-conjugated anti-B220 (clone RA3-6B2); peridinin chlorophyll protein-conjugated (PerCP) anti-CD3ε (clone 145-2C11); Fluorescein isothiocyanate-conjugated (FITC) anti-CD3ε (clone 17A2); PE- or PerCp-conjugated anti-CD4 (clone L3T4); PerCp-, FITC or allophycocyanin-conjugated anti-CD8 (clone Ly-2); PE- or allophycocyanin-conjugated anti-CD62L (clone MEL-14). Antibodies used from eBioscience: PE-conjugated anti-FoxP3 (clone NRRF-30); FITC-conjugated anti-CD44 (clone IM7); FITC-conjugated anti-CD27 (clone LG.7F9). Intracellular stainings for FoxP3 were performed using Foxp3 Fixation/Permeabilization Concentrate and Diluent (eBioscience), according to the manufactures protocol. Data were collected on a FACSCalibur or FACSCanto (Becton Dickinson) and were analyzed with FlowJo software (Treestar, Inc.).

2.3. T cell stimulation assays

2.3.1. Direct ex vivo cytokine production

Splenocytes were plated at 1×10^6 cells/well in a 96-well round-bottom plate and stimulated for 6 h with 1 ng/ml PMA and 1 μM ionomycin, of which the last 4 h was in the presence of 1 μg/ml Brefeldin A (Sigma). Hereafter, cells were stained for CD4 and CD8 followed by fixation and permeabilization. Cells were then incubated for 30 min with fluorescent labelled antibodies against either IFN-γ, IL-17, IL-4, IL-5, IL-13 (eBioscience/BD).

2.3.2. T_H cell polarization

Naïve ($CD44^-CD62L^+$) $CD4^+$ T cells and B ($B220^+$) cells were electronically gated and sorted using a FACSAria cell sorter (Becton Dickinson). The purity of cells sorted using this method was consistently >96%. Sort purified naïve $CD4^+$ T cells from WT mice were then stimulated for 3 days under T_H0, T_H1 or T_H17 polarizing conditions, or 7 days under T_H2 polarizing conditions, in the presence of WT or CD70TG derived B cells in a 1:1 ratio. All T cell polarization conditions included plate-bound αCD3 (clone 145-2C11, 5 μg/ml), soluble αCD28 (clone PV-1, 1 μg/ml) (both a kind gift from Dr. Louis Boon, Bioceros, The Netherlands) and soluble IL-2 (25 ng/ml) (Invitrogen). For T_H1 polarization 10 ng/ml IL-12 (R&D Systems) and 5 μg/ml αIL-4 (clone 11B11, a kind gift from Louis Boon, Bioceros) was added. For T_H2 polarization 50 ng/ml IL-4 (R&D Systems), 5 μg/ml αIL-12 (clone c17.8) and 20 μg/ml αIFN-γ (clone XMG 1.2, both mAbs were a kind gift from Louis Boon, Bioceros) was added. For T_H17 polarization, 3 ng/ml TGF-β (R&D Systems) and 20 ng/ml IL-6 (Peprotech) was included. At the end of the culture period, cells were washed and stimulated with 1 μM ionomycin, 1 ng/ml PMA and 1 μg/ml Brefeldin A for 5 h. Cells were then stained for CD4 and CD8 followed by fixation and permeabilization and stained for IL-4, IL-5, IFN-γ, IL-17, IL-10 and IL-13 as described above.