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Rapid Communication

Inflammation-inducing Th1 and Th17 cells differ in their expression patterns of apoptosis-related molecules

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ABSTRACT

Th1 cells are remarkably more susceptible to activation induced cell death than Th17. Here, we compared cultures of these two cell subpopulations for their expression of apoptosis-related molecules when re-exposed to their specific antigen. We also compared the expression of apoptosis-related molecules in the mouse eye with inflammation induced by Th1 or Th17 cells. Using qPCR we found that the mRNA transcript levels of the majority of tested apoptosis-related molecules were higher in the Th1 cultures, and in eyes with Th1-induced inflammation. Apoptotic intrinsic pathway molecules played minor roles in the processes in vitro or in vivo, whereas extrinsic pathway molecules, as well as PD-1, its ligands and Tim3, were heavily involved.

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1. Introduction

Analysis of the pathogenic processes of autoimmune diseases has established the involvement of the two major T-helper populations, Th1 and Th17 [1–4]. The two populations differ in their mechanism of generation, cytokine production and physiological function [1–4]. In addition, we and other authors found that Th1 and Th17 cells also differ in their plasticity [5–7] and more recently, we demonstrated that immunopathogenic processes mediated by Th17 cells are more sustainable than those mediated by Th1 cells [8]. Further, our data [8] and those by Yu et al. [9] suggested that the difference in sustainability of inflammation induced by Th1 and Th17 cell lines is related to the latter cell population being more resistant to activation-induced cell death (AICD,[1] also named "restimulation-induced cell death"). These two studies also suggested that the resistance to AICD by Th17 cells is due to lower production of Fas-ligand (FasL), as compared to Th1 cells [8,9].

Apoptosis, or programmed cell death, plays crucial roles in the immune system, in particular at the selection processes in the thymus [10,11] and during the immune response in the periphery, when the majority of antigen-specific lymphocytes should be eliminated at the end of immunization cycle, by the AICD process [12,13]. AICD has been investigated mainly in vitro, in studies in which pre-sensitized cells undergo apoptosis following re-exposure to their target antigen [12,13]; less is known about this process in vivo.

Data collected in recent years revealed that apoptosis is induced by two mechanisms, the intrinsic ("mitochondrial") and extrinsic ("death receptor") pathways, with different families of molecules initiating each of the two pathways [14,15]. The intrinsic pathway, that is triggered by intracellular events, is mediated mainly by molecules that belong to the Bcl-2 family of proteins, that includes both pro-apoptotic molecules such as Bim, Bax, Bak and Bad and anti-apoptotic molecules, represented by Bcl-2 [16,17]. The extrinsic pathway is triggered by extracellular signals that bind to cellular pro-apoptotic ("death") receptors. Abundant information has been collected in particular concerning the activities of two death-receptor pathways, FasL and its receptor, Fas, and TRAIL and its multiple cellular receptors [18,19]. In addition to the mentioned apoptosis-related molecules of the two pathways, recent studies identified regulatory molecules whose expression leads cells to stages of exhaustion and even death. This group of molecules includes programmed death-1 (PD-1) and its ligands PD-L1 and PD-L2 [20,21], as well as Tim-3 [22].

Here, we compared the expression of various apoptosis-related molecules by Th1 and Th17 cells following antigenic re-stimulation in vitro and the inflammatory process they elicit in their target tissue in vivo.

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[1] *Abbreviations*: AICD, activation-induced cell death; FasL, Fas-ligand; PD-1, Programmed cell death-1; HEL, hen egg lysozyme; DC, dendritic cells; Tg, transgenic; APC, antigen presenting cells; qPCR, quantitative real-time PCR.

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2. Materials and methods

2.1. Mice

All mice used in this study were (FVB/N × B10.BR) F1 hybrids, transgenically expressing either hen egg lysozyme (HEL) in their eyes ("HEL-Tg"), or HEL-specific TCR by their T-cells ("3A9"); see Refs. [1,7] for details. The mice were housed in a pathogen-free facility and all manipulations were performed in compliance with the NIH Resolution on the Use of Animals in Research.

2.2. Reagents

In addition to reagents detailed in Refs. [1,7], all tested gene expression assays were obtained from Applied Biosystems.

2.3. Generation of cell lines

Th1 and Th17 cell lines were generated by the procedures detailed in Refs. [1,7]. All line cells used in the present study were collected following reactivation, as detailed in the mentioned references.

2.4. AICD assay

The cell death assay procedure detailed in Ref. [8] was used with one modification: the CD4 cultures were stimulated here with increasing concentrations of the specific antigen, hen egg lysozyme (HEL), as indicated, in the presence of $CD11c^+$ dendritic cells (DC), serving as antigen presenting cells (APC), from wild type F1 mice at a ratio of 5:1 (CD4:DC).

2.5. Induction of ocular inflammation by adoptive transfer of Th1 or Th17 cells

Activated Th1 or Th17 3A9 cells (5×10^6) were injected via the tail vein into naïve HEL-transgenic (Tg) mice and the recipients were sacrificed 5 days later. All eyes developed inflammation [1,7,8] and were used to extract mRNA for performing quantitative real-time PCR (qPCR).

2.6. qPCR

Transcript levels of the tested genes were assessed by qPCR. β-Actin was used as an endogenous control in all samples. Relative expression was calculated according to the manufacturer's instructions (Applied Biosystem), as described elsewhere [7].

2.7. Statistical analysis

Data were shown as mean ± SEM. The software GraphPad Prism was used to perform the statistical analyses of the data with two-tailed Student's t test. Significant differences were indicated as follows: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

3. Results

3.1. Th1 cells are more susceptible to AICD than Th17 cells

We have previously shown that Th1 cells are more susceptible than Th17 cells to AICD [8]. The apoptosis-inducing reactivation mechanism in the cited study was by the non-physiological anti-CD3 antibody and we confirmed here the observation by reactivating cells of the two lineages by the physiological mechanism, i.e., exposure to increasing concentrations of the specific antigen, HEL. The data of a representative experiment are summarized in Fig. 1 and show that Th1 cells undergo apoptosis at a rate remarkably faster than that of Th17 cells.

3.2. Th1 and Th17 cells reactivated in vitro differ in their expression profile of apoptosis-related molecules

To learn about the expression of apoptosis-related molecules by Th1 and Th17 cells that undergo reactivation in culture with the specific antigen, HEL, we extracted RNA from these cells and evaluated by qPCR their expression levels of mRNA transcripts of major molecules involved in the apoptosis process. RNA samples from $CD4^+$ cells of naïve mice were used for controls. The tested molecules belong to several families of molecules, including the intrinsic and extrinsic apoptosis pathways, as well as PD-1, its two ligands and Tim-3. Fig. 2A summarizes combined data of four experiments. Reactivated Th17 cells expressed significantly higher levels of the "pro-life" Bcl-2 transcript than did their Th1 cell counterpart, but no significant differences were noted between cells of the two lineages in their expression levels of pro-apoptotic Bad, Bak and Bax. Unlike the relative similarity between Th1 and Th17 cells in their expression of the three mentioned intrinsic pathway transcripts, cells of the two lineages differed remarkably in their expression of the other tested transcripts (Fig. 2A). Of these other seven tested transcripts, only one, of Fas, was expressed by Th17 at levels higher than those by Th1 cells (the difference was not statistically significant). In contrast, transcripts of Fas-L, TRAIL and Tim3 were expressed by Th1 at levels significantly higher than those expressed by Th17 cells. It is also of note that the expression levels of PD-L1 and PD-L2 transcripts of all three tested RNA samples were marginal.

3.3. Differences in transcript expression profile between eyes with inflammation induced by Th1 or Th17 cells

Adoptively transferred activated Th1 or Th17 cells specific toward HEL induce ocular inflammation in syngeneic recipient mice expressing HEL in their eyes [1,7,8]. As recorded in detail in Ref. [8], the onset of ocular inflammation induced by Th1 cells is on day 3 post-cell injection, while that of Th17 cells is on day 4. Yet, the inflammatory process is at its peak on day 5 and the inflammatory changes are similar in recipients of cells of the two lineages. As shown by figures in the cited study, the inflammatory process is "panuveitic" and affects essentially all eye components. It mainly includes infiltration of inflammatory cells into most ocular tissues and is also characterized by proteinaceous exudate in the anterior chamber and vitreous.

Adoptively transferred Th cells activated in vitro undergo re-activation upon exposure to their target antigen in the recipient tissue and initiate an inflammatory response. To examine the

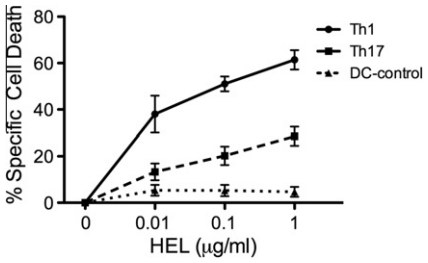

Fig. 1. Th1 cells are more susceptible to AICD than Th17 cells. Activated and polarized cells of the two lines were re-exposed to HEL at the indicated concentrations, in the presence of DC, and the % specific cell death was determined by the annexin/propidium iodide staining, as detailed in Ref. [8]. The apoptotic levels in control cultures, of DC alone, are also recorded in the figure.