JLB

Article

Viral dsRNA-activated human dendritic cells produce IL-27, which selectively promotes cytotoxicity in naïve CD8$^+$ T cells

Rosa de Groot,[1] Astrid J. van Beelen,[1] Ghaith Bakdash, Esther W. M. Taanman-Kueter, Esther C. de Jong,[2] and Martien L. Kapsenberg[2,3]

Department of Cell Biology and Histology, Academic Medical Center, University of Amsterdam, The Netherlands

RECEIVED JANUARY 31, 2012; REVISED MAY 16, 2012; ACCEPTED MAY 22, 2012. DOI: 10.1189/jlb.0112045

ABSTRACT

Viral recognition programs DCs to express Signal 3 molecules that promote the differentiation of effector CD8$^+$ T cells. Besides IL-12, another DC-derived IL-12 family member, IL-27, has been reported to contribute herein, but its specific role is not well understood. Here, we show that whereas IL-12 potently induces inflammatory cytokines (i.e., IFN-γ and TNF-α, but not IL-2), IL-27 excels in inducing proliferation and a cytotoxic profile (GrB, cytotoxicity of target cells) in human naïve CD8$^+$ T cells. Compared with bacterial cell-wall peptidoglycan, viral dsRNA-mimic poly (I:C) is superior in priming human BDCA1$^+$ peripheral blood DCs to produce IL-12 and IL-27, which promote inflammatory cytokines and a cytotoxic profile in differentiating CD8$^+$ T cells, respectively. These data support the concept that viral dsRNA-activated human DCs produce IL-27 to act as a specialized procytotoxic, antiviral cytokine in the development of effector CD8$^+$ T cells. *J. Leukoc. Biol.* 92: 605–610; 2012.

Introduction

DCs assess invasion of pathogens through PRRs that recognize structures associated with certain classes of pathogens. For example, cell surface PRRs recognize a variety of molecules associated with extracellular bacteria, whereas endosomal and cytoplasmic PRRs recognize ds- or ssRNA or -DNA from intracellular bacteria and viruses [1]. An important function of DCs is to produce Signal 3 molecules that tailor effector T cell-mediated immunity to the class of invading pathogen. In case of virus infection, DCs call for the expansion of CD8$^+$ T cells with an enhanced ability to kill infected target cells and to produce IFN-γ. DC-derived IL-12 has classically been regarded as a key third signal-cytokine for the development of effector CD8$^+$ T cells [2]. However, humans deficient in the IL-12 and IL-23 subunit p40 or the β1R subunit of the IL-12R and IL-23R may suffer from reduced protection against mycobacterium and certain intracellular bacteria, but they generally do not have problems defying viruses [3]. This suggests that in antiviral immunity, IL-12 and IL-23 are redundant, and their roles may be replaced by other molecules. Among more recently defined DC-derived factors contributing to development of CD8$^+$ T cell responses is the IL-12 family cytokine IL-27, which was originally described to induce IFN-γ in naïve CD4$^+$ T cells in synergy with IL-12 [4, 5]. A large series of studies have shown that whereas IL-27 in this way favors early Th1 differentiation, IL-27R-deficient mice exhibit enhanced, long-term Th1 responses after microbial infection and enhanced Th17 development, resulting in exacerbated encephalomyelitis of autoimmune or infectious origin (reviewed in refs. [6, 7]). This IL-27-mediated suppression of Th1/Th17-mediated pathology is explained by the ability of IL-27, in concert with TGF-β, to induce in CD4$^+$ and CD8$^+$ T cells the production of IL-10 [6, 7], which blocks inflammatory cytokines in myeloid cells and Th17 cells [8, 9].

In case of virus infection, effector T cell-derived IL-10 largely controls the magnitude of inflammation and tissue injury during acute virus infection [10]. However, at the same time, IL-10 affects CD8$^+$ T cell responses probably, mainly by promoting and preserving the fate of memory precursor CD8$^+$ T cells [11]. These data imply that IL-27, apart from being anti-inflammatory, may have a profound antiviral function. Indeed, innate IL-27 effectively inhibits replication of HIV [12] and hepatitis C virus [13] in human T cells and macrophages. Moreover, IL-27 drives adaptive antiviral immunity by amplifying proliferation, IFN-γ production, and/or cytotoxicity in CD8$^+$ T cells [14–17]. The amount of IL-12 and IL-27 produced by DCs varies according to the mode of DC stimulation

Abbreviations: APC=allophycocyanin, BDCA1=blood DC antigen 1, EBI3=EBV-induced gene 3, GrB=granzyme B, IFN-I=type I IFN, IFN-λ=type III IFN, IL-12β2=IL-12Rβ2 chain, IRF3=IFN regulatory factor 3, poly (I:C)=polyinosinic:polycytidylic acid, SEB=Staphylococcus aureus enterotoxin B

The online version of this paper, found at www.jleukbio.org, includes supplemental information.

1. These authors contributed equally as first authors.
2. These authors contributed equally as last authors.
3. Correspondence: Dept. of Cell Biology and Histology, Academic Medical Center, University of Amsterdam, Meibergdreef 15, 1105-AZ Amsterdam, The Netherlands. E-mail: m.l.kapsenberg@amc.uva.nl

0741-5400/12/0092-605 © Society for Leukocyte Biology

and is determined predominantly by the levels of the IL-12p35 and IL-27p28 subunits, respectively. Previous studies have indicated that IL-12 and IL-27 are produced readily by DCs activated by poly (I:C) [18, 19], a synthetic mimic of viral dsRNA, and a TLR3 ligand that is crucial in the initiation of an antiviral $CD8^+$ T cell response [20, 21]. At present, it is unclear to what extent and in what way virus-stimulated DCs set the tone for $CD8^+$ T cell development. We here show that viral dsRNA programs DCs to produce IL-12 and IL-27 that exert different effector functions in developing $CD8^+$ T cells.

MATERIALS AND METHODS

Reagents

Human rIL-12 and rIL-27 were purchased from Strathmann (Germany) and R&D Systems (Minneapolis, MN, USA), respectively. Neutralizing mAb to human IL-12 (20C2) and control antibodies were purchased from U-Cytech Biosciences (The Netherlands). Blocking antibodies against *CD130* (B-T2) and control antibodies were purchased from Gen-Probe Diaclone SAS (France). All human cultures were performed in IMDM (Life Technologies, Carlsbad, CA, USA), containing 10% FCS (HyClone, Thermo Scientific, Logan, UT, USA), supplemented with gentamycin (86 μg/ml, Duchefa, The Netherlands).

Isolation of human naïve $CD8^+$ T cells and DCs

Isolation of human naïve $CD8^+$ T cells. Untouched $CD8^+$ T cells were isolated from PBMCs to high purity (>98%, as assessed by flow cytometry) using a MACS isolation kit (Miltenyi Biotec, Germany). Next, naïve $CD8^+$ T cells were FACS-sorted on a FACSAria (BD Biosciences, San Jose, CA, USA) using CD27-PE, CD45RA-FITC, CD45RO-APC, and CD8-APC-Cy7 (BD Biosciences).

Isolation of $BDCA1^+$ DCs. PBMCs from healthy volunteers were enriched by Percoll gradient centrifugation, and $BDCA1^+$ DCs were isolated form PBMCs to high purity (>98%, as assessed by flow cytometry; Supplemental Fig. 1) by FACS-sorting using BDCA1 (BD Biosciences) and the lineage markers CD3, CD14, CD16, CD19, CD20, and CD56, BDCA1-APC (Miltenyi Biotec).

Cytokine production by $BDCA1^+$ DCs

$BDCA1^+$ (5×10^4 cells) DCs were stimulated with 10 μg/ml PGN (Sigma, St. Louis, MO, USA) or 20 μg/ml poly (I:C) (Sigma) in 96-well round-bottom culture plates (Costar, Corning, NY, USA). Supernatants were harvested after 24 h and stored at −20°C until the levels of IL-12, IL-27, IL-6, IL-1β, and TNF-α secretion were measured by sandwich ELISA, as described previously [22] or according to the manufacturer's protocol (IL-27 ELISA kit, eBioscience, San Diego, CA, USA).

In vitro activation of DC and T cell assays

After $BDCA1^+$ DCs were stimulated as described above for 24 h, DCs were cocultured with sorted, naïve $CD8^+$ T cells at a ratio of 1:4 (5000 $BDCA1^+$ DCs with 20,000 T cells) in the presence of 1 pg/ml superantigen SEB (Sigma). In cultures without DCs, naïve $CD8^+$ T cells were stimulated with plate-bound anti-human CD3 (16A9; Sanquin, The Netherlands; 0.1 μg/ml) and anti-human-CD28 (15E8; Sanquin; 0.1 μg/ml) in flat-bottom plates (Corning, Corning, NY, USA). Proliferation of cells was analyzed by overnight ^{3}H-thymidine incorporation at Day 3. Expression of GrB was measured by intracellular staining using anti-GrB-PE (Sanquin) on cells fixated with 3.7% paraformaldehyde after saponine permeabilization. For intracellular cytokine staining, cells were restimulated on Day 6 for 4 h with PMA/ionomycin in the presence of Brefeldin A and after fixation and permeabilization, stained with IFN-γ-PE (BD Biosciences), TNF-α-APC (BD Biosciences), and IL-2-FITC (BD Biosciences) and analyzed by FACS, as described previously [22]. To determine cytokine secretion, 1×10^5 cells were restimulated on Day 6 for 24 h with anti-human-CD3 (16A9; Sanquin; 0.1 μg/ml) and anti-human-CD28 (15E8; Sanquin; 0.1 μg/ml) in round-bottom plates (Corning). Supernatants were harvested and stored at −20°C until the levels of IL-2, IFN-γ, and TNF-α secretion were measured by sandwich ELISA, as described previously [22]. Cytotoxicity was measured by chromium release assay, as described before [23], using as target cells an EBV-transformed B cell line JY labeled with ^{51}Cr (Perkin Elmer, Waltham, MA, USA), loaded with SEB (5 ng/ml).

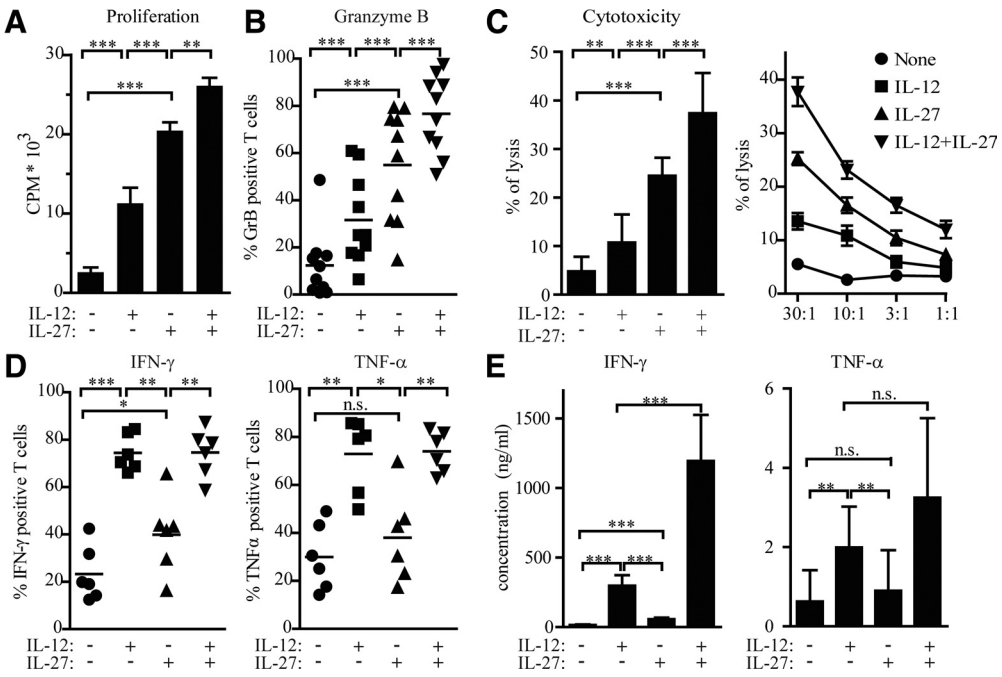


Figure 1. IL-12 and IL-27 act as third signal molecules for $CD8^+$ T cells. Naïve $CD8^+$ T cells were activated with plate-bound anti-CD3 and anti-CD28 in the presence of recombinant cytokines IL-12 (10 ng/ml), IL-27 (30 ng/ml), or both (as indicated in the figure). After 3 days, proliferation (A) and GrB expression (B) were determined. The capacity of the $CD8^+$ T cells to kill target cells was tested in a ^{51}Cr release assay at Day 5 (C). Pooled data of three independent experiments are shown at an E:T ratio of 30:1 (left panel), together with a representative experiment for all ratios tested (right panel). After 6 days, cells were restimulated for 4 h, and the frequency of intracellular IFN-γ- and TNF-α-expressing T cells was determined by FACS (D). Alternatively, after 6 days, cells were restimulated for 24 h to determine IFN-γ and TNF-α secretion by ELISA (E). Data are shown as means ± SD of three (A, C, and E) or more (B and D) independent experiments. $*P < 0.05$; $**P < 0.001$; $***P < 0.0001$.

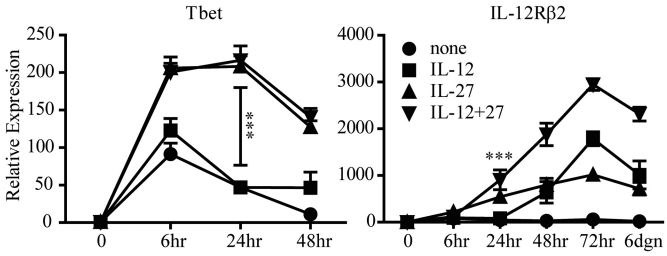


Figure 2. Induction of IL-12β and T-bet expression in naïve CD8+ T cells by IL-12 and IL-27. Naïve CD8+ T cells were activated with plate-bound anti-CD3 and anti-CD28 in the presence of recombinant cytokines IL-12 (10 ng/ml), IL-27 (30 ng/ml), or both (as indicated in the figure). After stimulation, cells were lysed at different time-points (as indicated in the figure), and relative expression of T-bet and IL-12β2 mRNA was measured by RT-PCR. At 24 h, significance was calculated between IL-12 and IL-27. Data are shown as means ± SD of three independent experiments. ***$P < 0.0001$.

Real-time quantitative RT-PCR analyses

Quantitative RT-PCR on DCs was performed using an iCycler (Bio-Rad, Hercules, CA, USA), based on specific primers and general SYBR Green (iQ Supermix, Bio-Rad) fluorescence detection, as described previously [24]. Prior to analysis, the concentration of mRNA was determined and adjusted. A panel of multiple appropriate reference genes was used, and data were analyzed using the ΔΔ comparative threshold method.

Primers used include: T-bet, forward CCT ACC AGA ATG CCG AGA TTA CTC; reverse GAT GCT GGT GTC AAC AGA TGT GTA C; IL-12Rβ, forward CAG GTG ACC TTG CAG GAG CT; reverse GTC GAC TCC GTA GCC AGT TTA; IL-12p40, forward ACG TTT CAC CTG CTG GTG GCT; reverse CTC CGC ACG TCA CCC CTT GG; IL-12p35, forward AGT GCC GGC TCA GCA TGT GTC; reverse GTG GCC ACG GGG AGG TTT CT; EBI3, forward CGT GCC TTT CAT AAC AGA GCA; reverse GAC GTA GTA CCT GGC TCG G; and IL-27p28, forward GCT CCC TGA TGT TTC CCT GAC C; reverse AAG GGC TGA AGC GTG GTG GA.

Statistical analysis

Where indicated, results from independent experiments were pooled and significance was calculated using a two-tailed paired Student's *t* test.

RESULTS

IL-12 and IL-27 differentially induce effector functions in human naïve CD8+ T cells

The role of IL-12 and IL-27 on CD8+ T cell development was assayed by adding increasing doses of recombinant cytokines to purified human naïve ($CD45RA^+CD45RO^-$) CD8+ T cells stimulated by the combination of anti-CD3 and anti-CD28 antibodies. In these experiments, IL-27 is steadily superior to IL-12 in inducing proliferation determined by thymidin incorporation (**Fig. 1A** and Supplemental Fig. 2A), in parallel to previous findings in naïve mouse T cells [16]. Moreover, we here show for the first time that IL-27 is highly superior to IL-12 in inducing expression of GrB, as determined by intracellular staining (Fig. 1B and Supplemental Fig. 2B), and cytotoxic activity, as determined by the nonspecific killing of target cells (Fig. 1C). In marked contrast, IL-12 appeared to be superior to IL-27 in inducing the capability to express IFN-γ and TNF-α (but not IL-2; data not shown) in the effector CD8+ T cells, both in terms of percentages of IFN-γ- and TNF-α-producing cells (Fig. 1D and Supplemental Fig. 2C) and secreted cytokines (Fig. 1E), in line with the well-known, proinflammatory role of IL-12 in CD4+ T cells [25]. In all of these assay types, the combination of IL-12 and IL-27 could be cumulative, but no substantial synergism was found (Supplemental Fig. 1A–C), except for secreted IFN-γ (Fig. 1E). Altogether, these experiments indicate a striking specialization of IL-12 and IL-27 in the programming of naïve CD8+ T cells. Whereas IL-12 mainly imprints naïve CD8+ T cells to become inflammatory IFN-γ-producing effector CD8+ T cells, IL-27 predominantly induces cytotoxicity in CD8+ T cells. These data stress the general inflammatory nature of IL-12 and support the concept that IL-27 stands out as a procytotoxic, e.g., antiviral, cytokine.

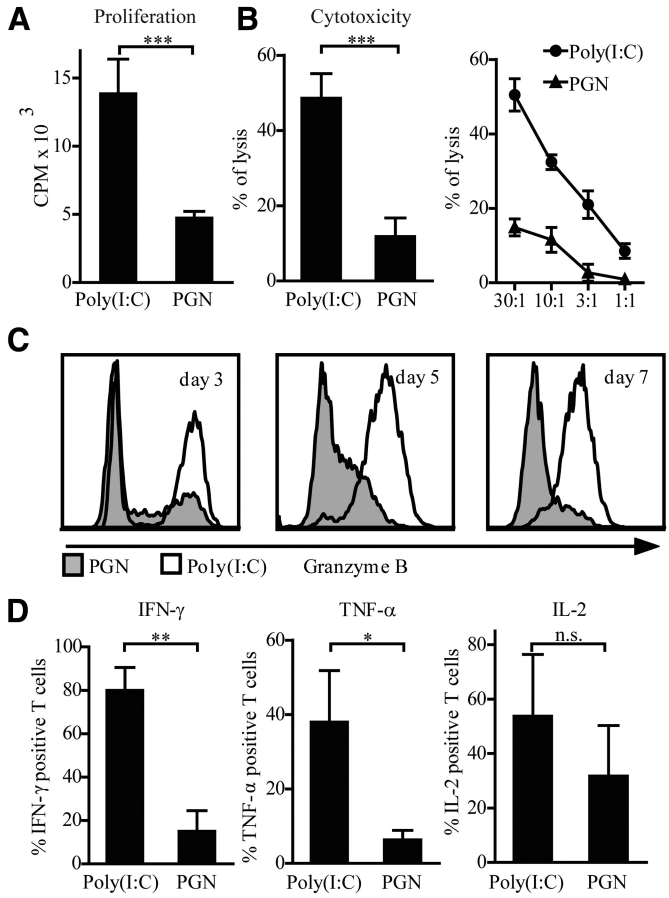


Figure 3. poly (I:C), but not PGN, promotes the capacity of BDCA1+ blood DCs to initiate cytotoxic CD8+ T cell responses. FACS-sorted BDCA1+ blood DCs were preactivated for 24 h in the presence of poly (I:C) and cocultured with naïve CD8+ T cells in the presence of SEB (1 pg/ml). Proliferation of CD8+ T cells was measured at Day 3 (A). GrB expression was determined at Days 3, 5, and 7 (C). Cytotoxicity was determined at Day 5 (B); pooled data of three independent experiments are shown at an E:T ratio of 30:1, besides a representative experiment for all ratios tested. After 6 days, the frequency of IFN-γ-, TNF-α-, and IL-2-producing T cells was measured by intracellular FACS staining (D). Data are shown as means ± SD of three (A and B) or more (D) independent experiments or as a representative of three independent experiments (C). *$P < 0.05$; **$P < 0.001$; ***$P < 0.0001$.

IL-27 induces transcription factor T-bet and IL-12β2 in mouse CD4$^+$ [5] and CD8$^+$ T cells [16]. We confirmed these data for human CD8$^+$ T cells and found that IL-27 is superior to IL-12 in this respect. However, addition of IL-27 enhanced transcription of the T-bet gene significantly more but transcription of the IL-12β2 gene considerably earlier than IL-12 (**Fig. 2**). IL-12 and IL-27 do not substantially regulate IL-12β1 and the two IL-27R chains (data not shown). The up-regulation of the expression of the IL-12β2, which is the IL-12R-specific chain [26], by IL-27 is in line with the observed synergy between IL-27 and IL-12 in the priming for IFN-γ production (Fig. 1E).

poly (I:C) primes BDCA1$^+$ DCs to promote effective cytotoxic CD8$^+$ T cell responses

As cytotoxic CD8$^+$ T cells are crucial in antiviral immunity, we subsequently studied to what extent virus preferentially programs DCs to produce IL-27 and to selectively induce cytotoxicity in developing naïve CD8$^+$ T cells. To evaluate the general concept that virus preferentially promotes CD8$^+$ T cell responses, human BDCA1$^+$ blood-derived DCs were primed ex vivo for 24 h with poly (I:C) or a bacterial cell wall component PGN and subsequently cocultured with allogeneic, naïve CD8$^+$ T cells. Clearly, poly (I:C)-primed DCs are superior compared with PGN-primed DCs in inducing proliferation of CD8$^+$ T cells (**Fig. 3A**), and these T cells contain markedly higher percentages of GrB-positive cells, as assessed by intracellular FACS staining (Fig. 3B). In line with enhanced GrB, these CD8$^+$ T cells exhibit an enhanced capacity to kill target cells (Fig. 3C). In addition, the CD8$^+$ T cells induced by the poly (I:C)-primed T cells harbor significantly higher percentages of cells expressing the effector cytokines IFN-γ and TNF-α but not IL-2 (Fig. 3D).

poly (I:C) primes BDCA1$^+$ DCs to produce high amounts of IL-12 and IL-27

Whereas the different CD8$^+$ T cell responses are not related to a different maturation status of the poly (I:C)- and PGN-primed DCs (data not shown), these DCs show markedly different cytokine profiles. PGN-primed DCs express more IL-1β, whereas poly (I:C)-primed DCs express more IL-6, IL-12p70, and IL-27 but express comparable levels of TNF-α (**Fig. 4A**) [24]. With regard to IL-12 and IL-27, poly (I:C)-primed DCs indeed show markedly enhanced expression of the critical IL-12/p35 and IL-27/p28 subunits, where poly (I:C) and PGN induced comparable levels of the subunits p40 and EBI3, which are shared with IL-23 and IL-35, respectively (Fig. 4B), further stressing that poly (I:C) is a potent inducer of IL-12 and IL-27.

poly (I:C)-primed DCs differentially use IL-12 and IL-27 to promote different effector functions in CD8$^+$ T cells

To study the relative contribution of IL-12 and IL-27 secreted by poly (I:C)-primed DCs on CD8$^+$ T cell effector functions, naïve CD8$^+$ T cells were activated with poly (I:C)-primed DCs in the presence of neutralizing antibodies against IL-12 and

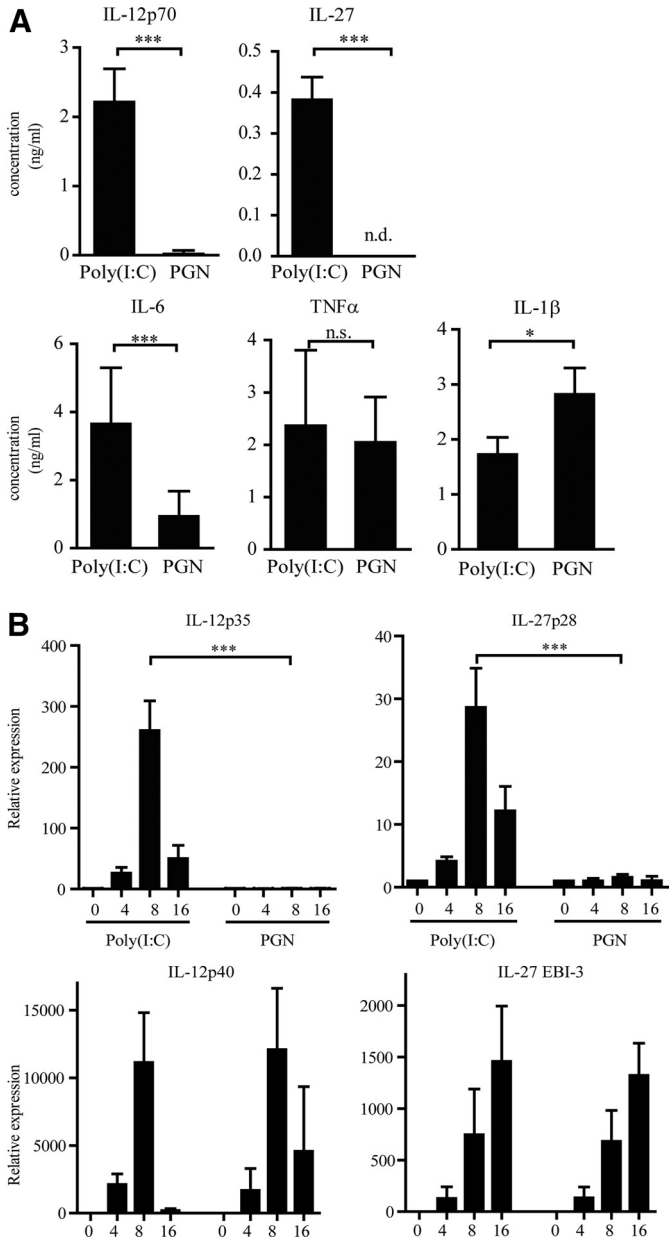


Figure 4. poly (I:C) and PGN induce differential cytokine expression patterns. FACS-sorted BDCA1$^+$ blood DCs (A and B) were activated with poly (I:C) for 24 h (A) or 0, 4, 8, and 16 h (B). Cytokine production (IL-6, TNF-α, IL-1β, IL-12p70, and IL-27) was measured by ELISA (A). IL-12p35, IL12p40, IL-27p28, and EBI3 mRNA levels were measured by quantitive RT-PCR (B). Data are shown as means ± SD of three independent experiments (A and B). $^*P < 0.05$; $^{***}P < 0.0001$.

IL-27R. Blocking of IL-27, but not blocking of IL-12, substantially inhibited the elevated GrB expression (**Fig. 5A**) and cytotoxicity (Fig. 5B), whereas the ability to produce IFN-γ was blocked significantly only by anti-IL-12 (Fig. 5C). The results of these experiments with primed DCs are in line with the data obtained with recombinant cytokines by showing that human DC-derived IL-27 mainly supports the development of cytotoxicity, and DC-derived IL-12 mainly supports the induction of IFN-γ in naïve CD8$^+$ T cells.

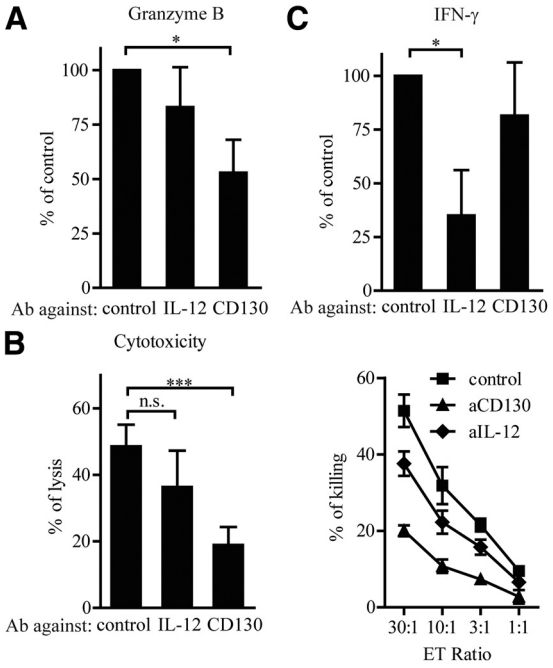


Figure 5. Relative contribution of IL-12 and IL-27 in CD8$^+$ T cell effector functions. poly (I:C)-activated BDCA1$^+$ blood DCs were cocultured with naïve CD8$^+$ T cells and neutralizing antibodies against IL-12, IL-27R (CD130), or control antibodies in the additional presence of SEB (1 pg/ml). Cells were analyzed at Day 3 for GrB expression (A). Cytotoxicity was determined at Day 5 (B); pooled data are shown for three independent experiments at an E:T ratio of 30:1, besides a representative experiment for all ratios tested. After 6 days, cells were restimulated, and the frequency of IFN-γ-expressing T cells was determined by FACS (C). Graphs show results of four independent experiments as mean percentage of control ± SD. $*P < 0.05$; $***P < 0.0001$.

DISCUSSION

Our data show that viral dsRNA programs DCs to produce IL-12 and IL-27, which differentially contribute to the development of effector CD8$^+$ T cells. Whereas IL-12 is important for the induction of cytokines, e.g., IFN-γ and TNF-α, IL-27 is superior to IL-12 in the induction of proliferation and effector functions, such as GrB expression and cytotoxicity in naïve CD8$^+$ T cells.

The selective ability of poly (I:C) to increase IL27p28 is in line with a previous report showing the crucial role of the IRF3 transcription factor in IL-27 synthesis by DCs [18]. Although poly (I:C) mainly activates endosomal TLR3 (associated with the adaptor, Toll-IL-1R domain-containing adaptor-inducing IFN-β), it may also activate cytosolic melanoma differentiation-associated gene-5 (associated with the adaptor, IFN-β promoter stimulator 1), both driving the activation of IRF3. Previous studies have indicated that IL-27 induces effector functions of mouse and human CD8$^+$ T cells [16, 17]. The present data detail this notion by showing that IL-27 is inferior to IL-12 in IFN-γ induction but largely superior to IL-12 in inducing cytotoxicity in human CD8$^+$ T cells, harnessing the concept that IL-27 acts as an antiviral cytokine. Interestingly, the production and function of IL-27 and IFN-I (i.e., IFN-α and IFN-β) are highly interconnected. The production of IL-27 and IFN-I in human DCs is readily induced by the triggering of viral sensors and depends on the activation of IRF1 and IRF3 [18], whereas IL-27 shares its innate [27] and CD8$^+$ T cell-mediated [28, 29] antiviral activity with IFN-I. It will be interesting to understand to what extent human DC subsets are specialized in enhanced production of IL-27, such as plasmacytoid DCs that produce high amounts of IFN-α and BDCA3$^+$ DCs that produce enhanced IFN-λ [30]. IL-27 additionally induces the anti-inflammatory cytokine IL-10 in CD4$^+$ and CD8$^+$ T cells, which dampens damage of Th1- and Th17-mediated inflammation during infection and autoimmunity (reviewed in ref. [6]), including acute virus infection [10]. On the other hand, IL-10 produced during acute virus infection may strongly limit CD4$^+$ T cell responses but does not interfere directly with effector and memory CD8$^+$ T cells [31].

The present data favor the concept that virus initiates cytotoxic CD8$^+$ T cell immunity by programming human DCs to produce IL-27. The close functional relationship between IL-27 and IFN-I (and probably IFN-λ) implies that IL-27 of IL-27R deficiencies may not readily demonstrate functional consequences for antiviral CD8$^+$ T cell-dependent immunity, such as in IL-27- or IL-27R-defient mice. However, the crucial importance of IL-27 in CD8$^+$ T cell-mediated immunity in vivo is underscored by the ability of endogenous and ectopic IL-27 to drive the development of protective CD8$^+$ T cells in different tumor models [32]. Further studies are required to better understand the condition of initiation and regulation of IL-27 production in specialized DC subsets and the way its adaptive antiviral function stands out compared with IFN-I and IFN-λ.

REFERENCES

1. Akira, S., Uematsu, S., Takeuchi, O. (2006) Pathogen recognition and innate immunity. *Cell* **124,** 783–801.
2. Mescher, M. F., Curtsinger, J. M., Agarwal, P., Casey, K. A., Gerner, M., Hammerbeck, C. D., Popescu, F., Xiao, Z. (2006) Signals required for programming effector and memory development by CD8+ T cells. *Immunol. Rev.* **211,** 81–92.
3. De Jong, J. R., Altare, F., Haagen, I. A., Elferink, D. G., Boer,T., van Breda Vriesman, P. J., Kabel, P. J., Draaisma, J. M., van Dissel, J. T., Kroon, F. P., Casanova, J. L., Ottenhoff, T. H. (1998) Severe mycobacterial and Salmonella infections in interleukin-12 receptor-deficient patients. *Science* **280,** 1435–1438.
4. Pflanz, S., Timans, J.C., Cheung, J., Rosales, R., Kanzler, H., Gilbert, J., Hibbert, L., Churakova, T., Travis, M., Vaisberg, E., Blumenschein, W. M., Mattson, J. D., Wagner, J. L., To, W., Zurawski, S., McClanahan, T. K., Gorman, D. M., Bazan, J. F., de Waal Malefyt, R., Rennick, D., Kastelein, R. A. (2002) IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4(+) T cells. *Immunity* **16,** 779–790.
5. Takeda, A., Hamano, S., Yamanaka, A., Hanada, T., Ishibashi, T., Mak, T. W., Yoshimura, A., Yoshida, H. (2003) Cutting edge: role of IL-27/WSX-1 signaling for induction of T-bet through activation of STAT1 during initial Th1 commitment. *J. Immunol.* **170,** 4886–4890.
6. Stumhofer, J. S., Hunter, C. A. (2008) Advances in understanding the anti-inflammatory properties of IL-27. *Immunol. Lett.* **117,** 123–130.
7. Yoshida, H., Miyazaki, Y. (2008) Regulation of immune responses by interleukin-27. *Immunol. Rev.* **226,** 234–247.
8. Chaudhry, A., Samstein, R. M., Treuting, P., Liang, Y., Pils, M. C., Heinrich, J. M., Jack, R. S., Wunderlich, F. T., Bruning, J. C., Müller, W., Rudensky, A. Y. (2011) Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. *Immunity* **34,** 566–578.
9. Huber, S., Gagliani, N., Esplugues, E., O'Connor Jr., W., Huber, F. J., Chaudhry, A., Kamanaka, M., Kobayashi, Y., Booth, C. J., Rudensky, A. Y., Roncarolo, M. G., Battaglia, M., Flavell, R. A. (2011) Th17 cells express interleukin-10 receptor and are controlled by Foxp3 and Foxp3+ regulatory CD4+ T cells in an interleukin-10-dependent manner. *Immunity* **34,** 554–565.

10. Sun, J., Madan, R., Karp, C. L., Braciale, T. J. (2009) Effector T cells control lung inflammation during acute influenza virus infection by producing IL-10. *Nat. Med.* **15,** 277–284.
11. Cui, W., Liu, Y., Weinstein, J. S., Craft, J., Kaech, S. M. (2011) An interleukin-21-interleukin-10-STAT3 pathway is critical for functional maturation of memory CD8+ T cells. *Immunity* **35,** 792–805.
12. Fakruddin, J. M., Lempicki, R. A., Gorelick, R. J., Yang, J., Adelsberger, J. W., Garcia-Pineres, A. J., Pinto, L. A., Lane, H. C., Imamichi, T. (2007) Noninfectious papilloma virus-like particles inhibit HIV-1 replication: implications for immune control of HIV-1 infection by IL-27. *Blood* **109,** 1841–1849.
13. Frank, A. C., Zhang, X., Katsounas, A., Bharucha, J. P., Kottilil, S., Imamichi, T. (2010) Interleukin-27, an anti-HIV-1 cytokine, inhibits replication of hepatitis C virus. *J. Interferon Cytokine Res.* **30,** 427–431.
14. Brender, C., Tannahill, G. M., Jenkins, B. J., Fletcher, J., Columbus, R., Saris, C. J., Ernst, M., Nicola, N. A., Hilton, D. J., Alexander, W. S., Starr, R. (2007) Suppressor of cytokine signaling 3 regulates CD8 T-cell proliferation by inhibition of interleukins 6 and 27. *Blood* **110,** 2528–2536.
15. Matsui, M., Moriya, O., Belladonna, M. L., Kamiya, S., Lemonnier, F. A., Yoshimoto, T., Akatsuka, T. (2004) Adjuvant activities of novel cytokines, interleukin-23 (IL-23) and IL-27, for induction of hepatitis C virus-specific cytotoxic T lymphocytes in HLA-A*0201 transgenic mice. *J. Virol.* **78,** 9093–9104.
16. Morishima, N., Owaki, T., Asakawa, M., Kamiya, S., Mizuguchi, J., Yoshimoto, T. (2005) Augmentation of effector CD8+ T cell generation with enhanced granzyme B expression by IL-27. *J. Immunol.* **175,** 1686–1693.
17. Schneider, R., Yaneva, T., Beauseigle, D., El-Khoury, L., Arbour, N. (2011) IL-27 increases the proliferation and effector functions of human naive CD8+ T lymphocytes and promotes their development into Tc1 cells. *Eur. J. Immunol.* **41,** 47–59.
18. Molle, C., Nguyen, M., Flamand, V., Renneson, J., Trottein, F., De Wit, D., Willems, F., Goldman, M., Goriely, S. (2007) IL-27 synthesis induced by TLR ligation critically depends on IFN regulatory factor 3. *J. Immunol.* **178,** 7607–7615.
19. Pichlmair, A., Reis e Sousa, C. (2007) Innate recognition of viruses. *Immunity* **27,** 370–383.
20. Salem, M. L., Kadima, A. N., Cole, D. J., Gillanders, W. E. (2005) Defining the antigen-specific T-cell response to vaccination and poly(I: C)/TLR3 signaling: evidence of enhanced primary and memory CD8 T-cell responses and antitumor immunity. *J. Immunother.* **28,** 220–228.
21. Schulz, O., Diebold, S. S., Chen, M., Naslund, T. I., Nolte, M. A., Alexopoulou, L., Azuma, Y. T., Flavell, R. A., Liljestrom, P., Reis e Sousa, C. (2005) Toll-like receptor 3 promotes cross-priming to virus-infected cells. *Nature* **433,** 887–892.
22. De Jong, E. C., Vieira, P. L., Kalinski, P., Schuitemaker, J. H., Tanaka, Y., Wierenga, E. A., Yazdanbakhsh, M., Kapsenberg, M. L. (2002) Microbial compounds selectively induce Th1 cell-promoting or Th2 cell-promoting dendritic cells in vitro with diverse th cell-polarizing signals. *J. Immunol.* **168,** 1704–1709.
23. Lotze, M. T., Grimm, E. A., Mazumder, A., Strausser, J. L., Rosenberg, S. A. (1981) Lysis of fresh and cultured autologous tumor by human lymphocytes cultured in T-cell growth factor. *Cancer Res.* **41,** 4420–4425.
24. Van Beelen, A. J., Zelinkova, Z., Taanman-Kueter, E. W., Muller, F. J., Hommes, D. W., Zaat, S. A., Kapsenberg, M. L., de Jong, E. C. (2007) Stimulation of the intracellular bacterial sensor NOD2 programs dendritic cells to promote interleukin-17 production in human memory T cells. *Immunity* **27,** 660–669.
25. Trinchieri, G. (2003) Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat. Rev. Immunol.* **3,** 133–146.
26. Rogge, L., Barberis-Maino, L., Biffi, M., Passini, N., Presky, D. H., Gubler, U., Sinigaglia, F. (1997) Selective expression of an interleukin-12 receptor component by human T helper 1 cells. *J. Exp. Med.* **185,** 825–831.
27. Remoli, M. E., Gafa, V., Giacomini, E., Severa, M., Lande, R., Coccia, E. M. (2007) IFN-β modulates the response to TLR stimulation in human DC: involvement of IFN regulatory factor-1 (IRF-1) in IL-27 gene expression. *Eur. J. Immunol.* **37,** 3499–3508.
28. Curtsinger, J. M., Lins, D. C., Johnson, C. M., Mescher, M. F. (2005) Signal 3 tolerant CD8 T cells degranulate in response to antigen but lack granzyme B to mediate cytolysis. *J. Immunol.* **175,** 4392–4399.
29. Le Bon, A., Durand, V., Kamphuis, E., Thompson, C., Bulfone-Paus, S., Rossmann, C., Kalinke, U., Tough, D. F. (2006) Direct stimulation of T cells by type I IFN enhances the CD8+ T cell response during cross-priming. *J. Immunol.* **176,** 4682–4689.
30. Lauterbach, H., Bathke, B., Gilles, S., Traidl-Hoffmann, C., Luber, C. A., Fejer, G., Freudenberg, M. A., Davey, G. M., Vremec, D., Kallies, A., Wu, L., Shortman, K., Chaplin, P., Suter, M., O'Keeffe, M., Hochrein, H. (2010) Mouse CD8α+ DCs and human BDCA3+ DCs are major producers of IFN-λ in response to poly IC. *J. Exp. Med.* **207,** 2703–2717.
31. Brooks, D. G., Walsh, K. B., Elsaesser, H., Oldstone, M. B. (2010) IL-10 directly suppresses CD4 but not CD8 T cell effector and memory responses following acute viral infection. *Proc. Natl. Acad. Sci. USA* **107,** 3018–3023.
32. Morishima, N., Mizoguchi, I., Okumura, M., Chiba, Y., Xu, M., Shimizu, M., Matsui, M., Mizuguchi, J., Yoshimoto, T. (2010) A pivotal role for interleukin-27 in CD8+ T cell functions and generation of cytotoxic T lymphocytes. *J. Biomed. Biotechnol.* **2010,** 605483.

KEY WORDS:
BDCA1+ DC · human cytokines · Granzyme B · IFN-gamma · Cytotoxic T Lymphocytes