Research Article

Journal of Innate Immunity

J Innate Immun
DOI: 10.1159/000350242

Received: September 3, 2012
Accepted after revision: February 26, 2013
Published online: ■■■

Impairment in Natural Killer Cells Editing of Immature Dendritic Cells by Infection with a Virulent *Trypanosoma cruzi* Population

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Key Words
Crosstalk · Cytotoxicity · Dendritic cells · Natural killer cells · Parasites · Protozoan · *Trypanosoma cruzi*

Abstract
Early interactions between natural killer (NK) and dendritic cells (DC) shape the immune response at the frontier of innate and adaptive immunity. Activated NK cells participate in maturation or deletion of DCs that remain immature. We previously demonstrated that infection with a high virulence (HV) population of the protozoan parasite *Trypanosoma cruzi* downmodulates DC maturation and T-cell activation capacity. Here, we evaluated the role of NK cells in regulating the maturation level of DCs. Shortly after infection with HV *T. cruzi*, DCs in poor maturation status begin to accumulate in mouse spleen. Although infection induces NK cell cytotoxicity and cytokine production, NK cells from mice infected with HV *T. cruzi* exhibit reduced ability to lyse and fail to induce maturation of bone marrow-derived immature DCs (iDCs). NK-mediated lysis of iDCs is restored by in vitro blockade of the IL-10 receptor during NK-DC interaction or when NK cells are obtained from *T. cruzi*-infected IL-10 knockout mice. These results suggest that infection with a virulent *T. cruzi* strain alters NK cell-mediated regulation of the adaptive immune response induced by DCs. This regulatory circuit where IL-10 appears to participate might lead to parasite persistence but can also limit the induction of a vigorous tissue-damaging T-cell response.

Introduction

Natural killer (NK) and dendritic cells (DCs) are critical actors of the early immune response. NK cells, a unique lymphocyte population of the innate immune response, play a central role in immune surveillance as a result of their cytokine production and cytolysis of infected or tumor cells. DCs stand at the frontier of innate and adaptive immunity. Chemokines, cytokines and microbial components promote their maturation and migration into lymphoid tissues where they activate the antigen-specific T-cell responses. The cooperative dialog between these cells during the innate immune response regulates the quality of the ongoing adaptive immune re-

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sponse against pathogens and tumors [1, 2]. Once DCs mature, they release cytokines such as IL-12 or IL-15 with the capacity to induce NK cell activation and proliferation [2]. Pathogen-primed NK cells secrete IFN-γ, TNF-α and GM-CSF, which can complete the process of DC maturation [2]. NK cells can optimize the conditions for T-cell activation by eliminating autologous immature DCs (iDCs) that have failed to mature properly while sparing mature DCs [1, 3–5]. This process adjusts the overall maturation level of the DC pool and appears to play a part in the balance towards immunogenic or regulatory responses [1, 6, 7].

Chagas' disease, caused by the protozoan parasite *Trypanosoma cruzi*, affects over 10 million people in Latin America [8]. The onset of human pathology is extremely diverse, varying from a relatively benign asymptomatic form to digestive compromise or a fatal cardiac course. Several lines of evidence indicate that the biological differences among *T. cruzi* isolates delineate the host-parasite relationship and disease outcome [9, 10]. The immune mechanisms triggered shortly after *T. cruzi* infection seem to be essential for the control of early parasite duplication. In this sense, NK cells play a role in parasite control, mainly by their early IFN-γ production [11–13]. However, NK are also able to kill extracellular parasites directly through contact-dependent mechanisms [14]. Humoral and cellular arms of the immune response are known to participate in the control of *T. cruzi* infection but fail to achieve total pathogen clearance. In addition, diverse studies show that *T. cruzi* infection elicits regulatory mechanisms able to curb immunity [15, 16]. In this line of work, we demonstrated in vivo and in vitro that *T. cruzi* and parasite-derived molecules can modulate DC maturation and function in a strain-dependent manner [17–19]. Thus, it is relevant to understand the participation of NK cells in the maturation or elimination of the iDCs in the context of *T. cruzi* infection. Therefore, we used experimental murine infection with two parasite populations displaying significant biological differences: RA is a highly virulent (HV), fast-duplicating, pantropic strain belonging to the TcVI lineage isolated from an acutely infected child [20] whereas K98 is a slow-duplicating, myotropic strain with low virulence (LV) belonging to TcI isolated from a chronically infected adult [21–24]. Here, we show that iDCs subsets start to accumulate in the spleen very early upon infection with the HV strain but not with the LV strain. Even though NK cells from mice infected with both parasite populations become functionally activated in a similar extent, NK cells from mice infected with the HV strain exhibit impaired deletion of iDCs and do not support their maturation. The regulatory cytokine IL-10, which is produced during in vivo *T. cruzi* infection, appears to participate in this regulatory mechanism involving NK cells and DCs.

Methods

Mice

C3H/HeN and Balb/c male mice were supplied by the animal facilities at the IMPaM (Universidad de Buenos Aires, CONICET) and IL-10 knock out (KO) male mice of Balb/c background by the animal facilities of the Fundación Instituto Leloir (Buenos Aires, Argentina). Mice were kept under standard conditions on a 12-hour light, 12-hour dark cycle in a temperature-controlled room (25 ± 2°C) with food and water ad libitum. All animal procedures were approved by institutional regulations of the Committee for the Care and Use of Laboratory Animals (approval No. RS2079/2007, Universidad de Buenos Aires) and in accordance with government regulations of the National Food Safety and Quality Service (SENASA, resolution No. RS617/2002, Argentina). All efforts were made to minimize the number of animals used and their suffering.

Parasites and Infection

Bloodstream forms (trypomastigotes) of the HV (RA) or the LV (K98) *T. cruzi* populations were maintained by serial passages in CF1 mice. C3H/HeN mice were infected with 15,000 trypomastigotes of either *T. cruzi* population inoculated into the hind footpad. Where indicated, IL-10KO male mice of Balb/c background and Balb/c controls were infected with the same inoculum by the same route. Sham-infected mice were inoculated with CF1 mouse blood. Parasitemia was monitored by counting the number of viable trypomastigotes in blood collected after lysis with 0.87% ammonium chloride buffer. Mouse survival was recorded daily.

Quantification of Tissue Parasite Burden by Quantitative PCR

In brief, tissue specimens were separately subjected to lysis in a buffer containing 50 mM Tris (pH 8; Promega, USA), 10 mM EDTA (Promega), 100 mM NaCl (Sigma, USA), 1% (w/v) SDS (Promega) and 300 μg/ml proteinase K (Promega). Samples were heated for 2 h at 55°C and DNA was purified by phenol extraction followed by ethanol precipitation. For PCR reaction, the *T. cruzi* satellite nuclear repeat was amplified with primers SatFw (5′-GCAGTCGGCKGATCGTTTTCG-3′) and SatRv (5′-TTCAG RGTTGTTTGGTGTCCAGTG-3′). The 20-μl reaction tube contained 0.5 μM of primers SatFw and SatRv, 3 mM $MgCl_2$, 250 μM of each dNTP, 0.5 U of platinum Taq polymerase (Invitrogen, Life Technologies, USA), SYBR Green (Invitrogen, Life Technologies) at a final concentration of 0.5× and 2 μl of sample DNA. Thermal cycling comprised an initial denaturation step for 5 min at 95°C, followed by 40 cycles at 94°C for 10 s, 65°C for 10 s and 72°C for 10 s on an MJR-Opticon II device (Promega). Host cell number was assessed by quantifying the single copy murine-specific TNF-α gene in a separate PCR reaction, using primers TNF-5241 (5′-TC CCTCTCATCAGTTCTATGGCCCA-3′) and TNF-5411 (5′-CA

GCAAGCATCTATGCACTTAGACCCC-3′) at a final concentration of 1 μM. All other PCR reagents and cycling conditions were the same as for the *T. cruzi* satellite DNA amplification. The number of parasites was referred to that of the host cells. The standard curve for host cell determination was generated with DNA extracted from serial 10-fold dilutions of previously quantified normal splenocyte cell suspensions. Standard curves for *T. cruzi* were generated with DNA extracted from splenocyte cell suspensions contaminated with 10-fold dilutions of *T.cruzi* epimastigotes. Epimastigotes from Silvio X10 (TcI) and CL-Brener (TcVI) *T. cruzi* strains were employed to standardize the amount of *T. cruzi* satellite DNA for K98 (TcI) and RA (TcVI) strains, respectively [25].

Cells

PBMC were isolated from blood samples by density gradient centrifugation using Histopaque-1083 (Sigma). Spleens were excised and homogenized with RPMI 1640 supplemented with 5% fetal bovine serum (FBS; Gibco-BRL, USA), 2-mercaptoethanol and antibiotics (penicillin 100 U/ml and streptomycin 100 μg/ml; Sigma). Erythrocytes were lysed and single cell suspensions were washed in RPMI 1640-10% FBS. Where needed, adherent mononuclear cells were depleted by incubation for 1 h at 37°C. The viability of cells was consistently over >98%.

Proliferation Assay

Excised spleens were homogenized and splenocytes (2.5×10^5) were cultured for 4 days at 37°C in RPMI 1640-10% FBS medium alone or with 5 μg/ml concanavalin A (Sigma) or 3 μg/ml anti-CD3 (Pharmingen, USA) at a final volume of 200 μl by triplicate. Cultures were pulsed with 1 μCi/well of [^{3}H]-thymidine (ICN, USA) during the last 24 h. Cells were harvested and analyzed in a Rack Beta liquid scintillation counter (Pharmacia, Sweden). Results were expressed as the mean count per minute (cpm) of culture ± SEM.

Purification of NK Cells

DX5+ cells were isolated from nonadherent spleen cell fractions by magnetic sorting using biotinylated anti-mouse CD49/pan-NK cell (DX5) monoclonal antibodies bound to streptavidin-coated magnetic beads and miniMACS columns (Miltenyi Biotech GmbH, Germany) according to the manufacturer's protocol. Cell populations were approximately 90% DX5+ cells by flow cytometry.

iDC Generation

iDCs were obtained by culturing bone marrow cells supplemented with 30% supernatant from a granulocyte-macrophage colony-stimulating factor (GM-CSF)-expressing cell line (J558 GM-CSF), as previously described [26].

Flow Cytometry

The following antibodies were used: anti-CD49b (DX5), anti-CD3 (145-2C11), anti-CD69 (H1.2F3), anti-CD11c (HL3), anti-IEk (11-5.2), anti-IAd (39-10-8), anti-CD80 (16-10A1), anti-CD86 (GL-1), anti-IFNγ (XGM.1) and anti-IL-10 (JES3–19F1) along with the appropriate isotype control antibodies (BD Biosciences or Caltag, UK). For intracellular cytokine staining, cells were incubated with brefeldin A (10 μg/ml; Sigma) for the last 4 h of culture. After surface staining, the cells were fixed in 4% paraformaldehyde (20 min), permeabilized [0.1% saponin (Sigma) in PBS 10% FBS] and stained intracellularly. Samples were acquired on a FACSCalibur (Becton Dickinson, USA), and data were analyzed with Cyflogic 1.2.1 free software (CyFlo Ltd, Finland).

CD107a Assay

Nonadherent cell fractions were resuspended at 1×10^6 cells/ml in RPMI 1640 supplemented with 10% FCS and incubated with FITC-conjugated anti-CD107a monoclonal antibody (BD Biosciences) for 1 h. Then, protein transport inhibitors were added to prevent intracellular degradation of internalized CD107a-antibody complexes (monensin, 6 μg/ml; BD Biosciences) and exocytosis (brefeldin A, 10 μg/ml). Cells were incubated for another 4 h at 37°C in 5% CO_2, washed with FACS buffer (PBS, 1% FCS, 1 mM EDTA, 0.05% sodium azide) and stained with the conjugated monoclonal antibodies anti-DX5 and anti-CD3.

5-(6)-Carboxyfluorescein Succinimidyl Ester-Based in vitro Cytotoxicity Assay

NK cell cytotoxicity against YAC-1 and iDC cells was evaluated in a 4-hour 5-(6)-carboxyfluorescein succinimidyl ester (CFSE)/propidium iodide (PI) flow-cytometric assay [27]. Briefly, target cells were labeled with CSFE (5 μM, Sigma) for 15 min at 37°C in the dark. Then, they were cocultured at different ratios with purified NK effector cells from *T. cruzi*-infected (18 h.p.i.) and control mice (5×10^4 target cells/well) in 96-well plates in a total volume of 200 μl RPMI 1640, 5% FCS/well. For in vitro blockade of IL-10R, 40 μg/ml of clone 1B1.3A or rat IgG isotype control (HPRN) from Santa Cruz Biotechnology (USA) were added at this step. After 4 h in a 5% CO_2 atmosphere at 37°C, PI was added (5 μM), and data were collected in a FACSCalibur (Becton Dickinson). Spontaneous (S) and maximum (M) cytotoxicity of target cells was determined in cultures with RPMI 1640-5% FCS and PBS-2% Tween-20 (Sigma), respectively. Percent NK cell activity was calculated as: (PI incorporation by experimental samples – S)/(M – S) × 100.

Statistics

Data were expressed as means ± SEM. The Mann-Whitney test and ANOVA followed by Bonferroni's post hoc test were employed for comparisons between two and more groups. Differences in survival between groups were analyzed by log-rank test. A value of $p < 0.05$ was considered significant. All tests were performed using the GraphPad prism version 5.00 for Windows (GraphPad, USA).

Results

Differences in Outcome of Experimental Infection with Two T. cruzi Populations of Divergent Virulence

LV (K98 *T. cruzi* strain)-infected mice presented low and delayed parasitemia levels as well as low splenic parasite burden. No mortality was registered during the acute phase of infection in this group of mice (fig. 1a–c). HV (RA *T. cruzi* strain)-infected mice exhibited elevated parasitemia levels; spleen parasite burden was 2–3 log units higher than with LV and only 50% of the mice reached the chronic phase of infection (fig. 1a–c). As re-

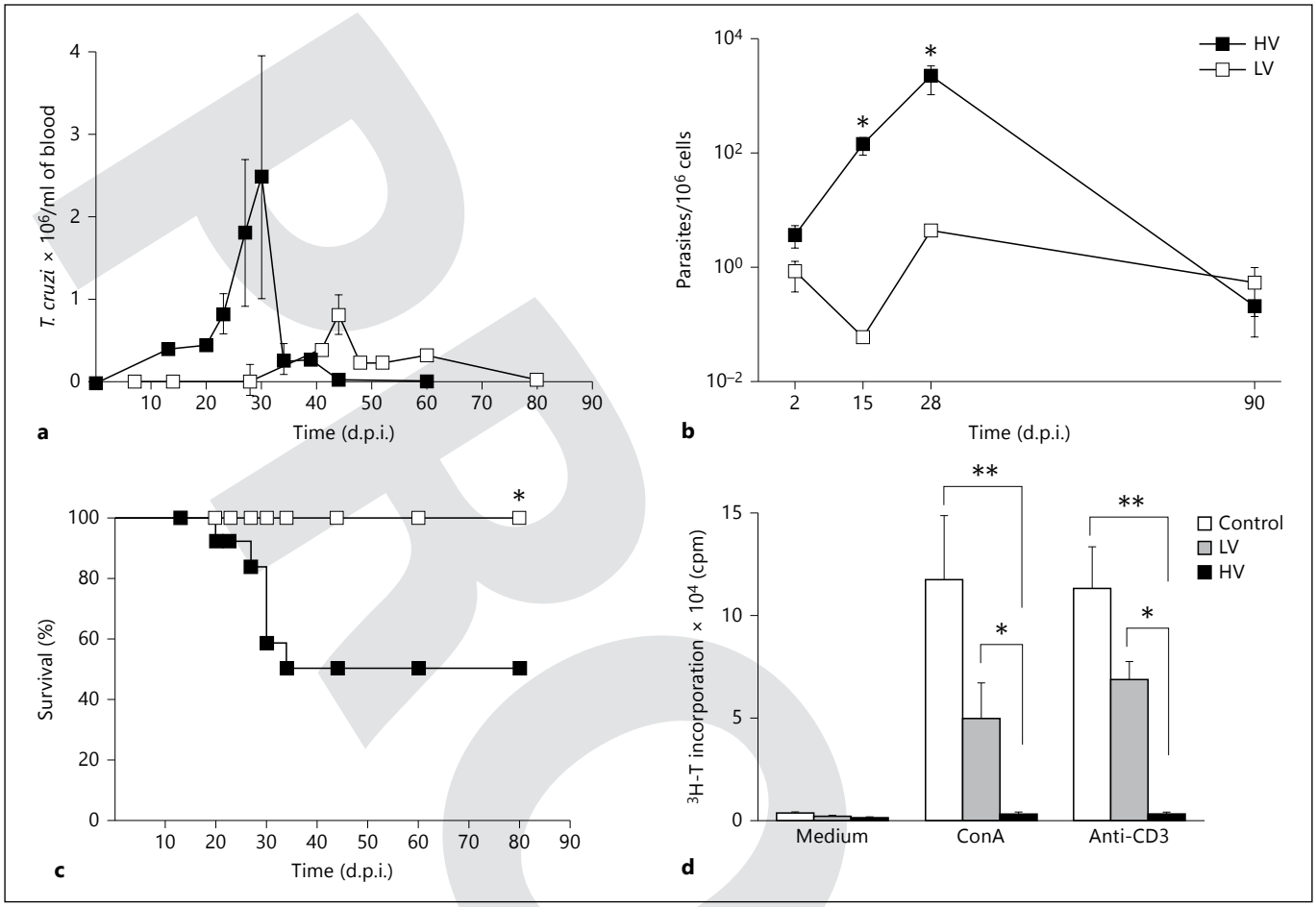

Fig. 1. Outcome of murine infection with the HV and LV *T. cruz*i populations. C3H/HeN mice were infected with HV and LV strains. Parasitemia levels (**a**), parasite burden in the spleen measured by quantitative PCR (**b**) and survival (**c**) were registered at the indicated time points. Splenocytes from acutely infected HV or LV *T. cruzi* strains (7 d.p.i.) and control mice were cultured with concanavalin A (ConA; 5 μg/ml) or anti-CD3 (3 μg/ml) or medium alone (**d**). Means ± SEM. One representative experiment of 2 using 6–10 individual mice per group is shown. **a**, **b** Mann-Whitney test. **c** log-rank (Mantel-Cox) test. **d** ANOVA (Bonferroni's post hoc test). * $p < 0.05$ and ** $p < 0.01$. T = Thymidine.

ported [28], splenocytes from mice acutely infected (7 days post-infection, d.p.i.) with *T. cruzi* displayed poor mitogen-induced proliferation compared to uninfected mice. However, those from mice infected with the HV strain showed significantly lower values than with the LV strain (fig. 1d). Proliferation was restored in HV- and LV-infected mice that reached the chronic phase of infection (90 d.p.i.; data not shown).

iDC Subsets Accumulate in the Spleen during Acute Infection with the HV T. cruzi Population

Total cell number was significantly increased in the spleen of HV- and LV-infected animals 15 and 28 d.p.i., respectively (fig. 2a). Next, two distinct DC subsets residing in the spleen were analyzed, a conventional CD11c high ($CD11c^{hi}$) DC subset and a CD11c intermediate ($CD11c^{int}$) semimature DC subset (fig. 2b). Following infection with the HV population, the frequency of the $CD11c^{int}$ DC subset started to increase (2 d.p.i.) and, later on, the absolute numbers of both $CD11c^{hi}$ and $CD11c^{int}$ DC subsets increased significantly in this group of mice compared to the one infected with the LV population (fig. 2c, d). Infection with the HV strain decreased significantly MHC class II expression at the surface of both $CD11c^{hi}$ and $CD11c^{int}$ DC subsets (fig. 2e, f). This phenomenon started early after infection (2 days) and lasted until the host reached the chronic stage of the disease (90 d.p.i.).

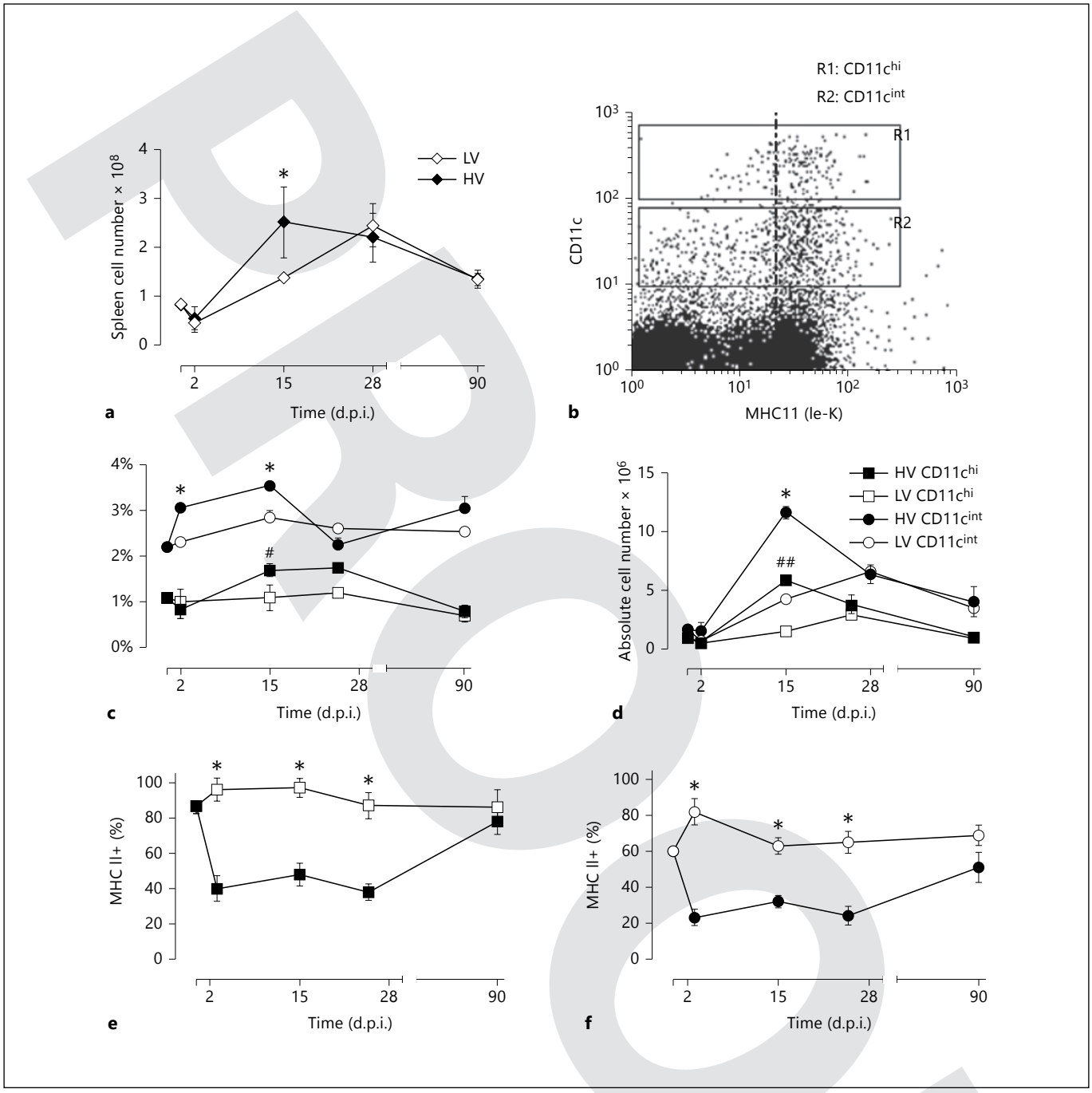

Fig. 2. Accumulation of immature splenic DC subsets during acute in vivo HV *T. cruzi* infection. **a** Total number of spleen cells in C3H/HeN mice infected with HV or LV *T. cruzi* populations at the indicated time points. **b** Dot plot representation of spleen cell suspensions from a control mouse stained for CD11c and MHC class II (Ie-K) surface markers. The dotted line represents the limit between Ie-K-positive and -negative cells. $CD11c^{hi}$ and $CD11c^{int}$ splenic DC subsets are plotted in R1 and R2 regions, respectively. Frequency (**c**) and absolute cell number (**d**) of $CD11c^{hi}$ and $CD11c^{int}$ splenic DC subsets from infected mice. Surface expression of MHC II (Ie-K) by the splenic $CD11c^{hi}$ DC subset (**e**) and by the $CD11c^{int}$ DC (**f**) from infected mice. Means ± SEM (**a**, **c, f**) of values obtained from 3 independent experiments with 3–5 pooled (**e**, **f**) or independent (**a**, **c**, **d**) mice per group. Mann-Whitney test. **a**, **e**, **f** * $p < 0.05$ HV vs. LV. **c**, **d** * $p < 0.05$ HV $CD11c^{int}$ vs. LV $CD11c^{int}$; # $p < 0.05$, ## $p < 0.01$ HV $CD11c^{hi}$ vs. LV $CD11c^{hi}$.

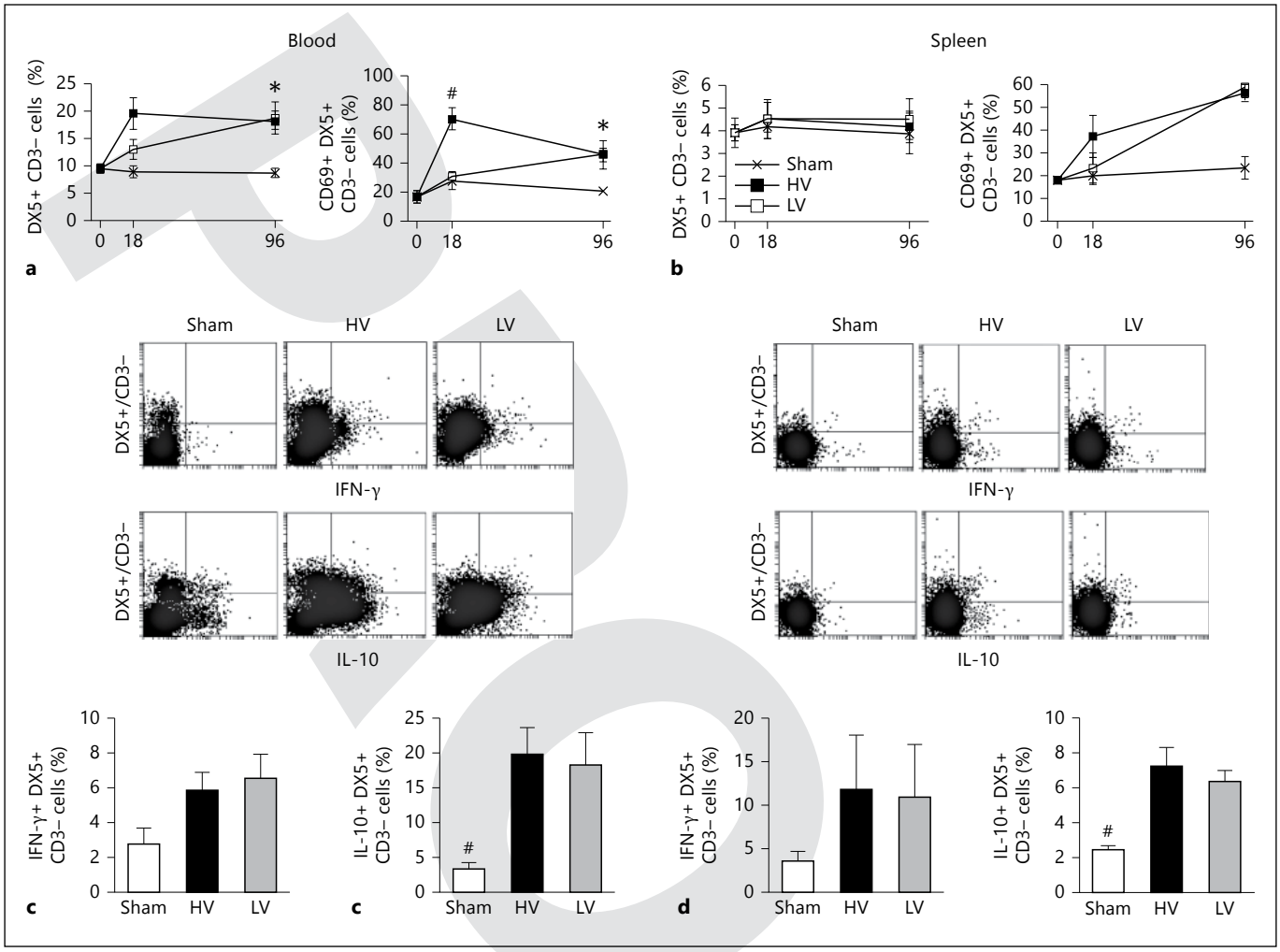

Fig. 3. Frequency, activation and cytokine production by NK cells following *T. cruzi* infection. C3H/HeN mice were infected with the HV and LV *T. cruzi* populations and sham infected. PBMC (blood) and spleen cell suspensions (spleen) obtained at different time points after infection. Frequency of DX5+/CD3– cells and expression of CD69 by DX5+/CD3– cells were evaluated in PMBC (**a**) and spleen cell suspensions (**b**). Ex vivo intracellular production of IFN-γ and IL-10 by DX5+/CD3– cells from PBMC fractions (**c**) and spleen cell suspensions (**d**) 18 h.p.i. Dot plots show results from 1 representative experiment of 3 each using a pool of 3–5 mice per group. Histograms show results obtained from 3 independent experiments with 3–5 pooled mice per group (means ± SEM). ANOVA (Bonferroni's post hoc test). * $p < 0.05$ sham vs. LV and HV, # $p < 0.05$ sham vs. HV and LV.

Early Infection with LV and HV T. cruzi Populations Increases Circulating NK Cell Frequency, Activation and Production of IFN-γ but Also of IL-10 Cytokines

Eighteen hours after infection with the HV *T. cruzi* population, the relative number and activation (CD69 expression) of circulating NK cells increased (fig. 3a). Circulating NK cells from mice infected with the LV population attained later (96 h.p.i.) the levels of those infected with the HV strain (fig. 3a). In the spleen, the relative number of NK cells was not modified during the first 96 h of *T. cruzi* infection while their CD69 expression increased significantly upon infection with both parasite populations (fig. 3b). Regarding cytokine production by NK cells, 18 h after infection with the HV and LV *T. cruzi* populations, there was an increase in the relative number of IFN-γ-positive NK cells (DX5+CD3–) from PBMC fractions and the spleen compared to sham-infected controls (fig. 3c, d). In addition, during early infection with both *T. cruzi* populations, the number of NK cells producing IL-10 also increased significantly at the same compartments (fig. 3c, d) but there was no simultaneous production of IFN-γ

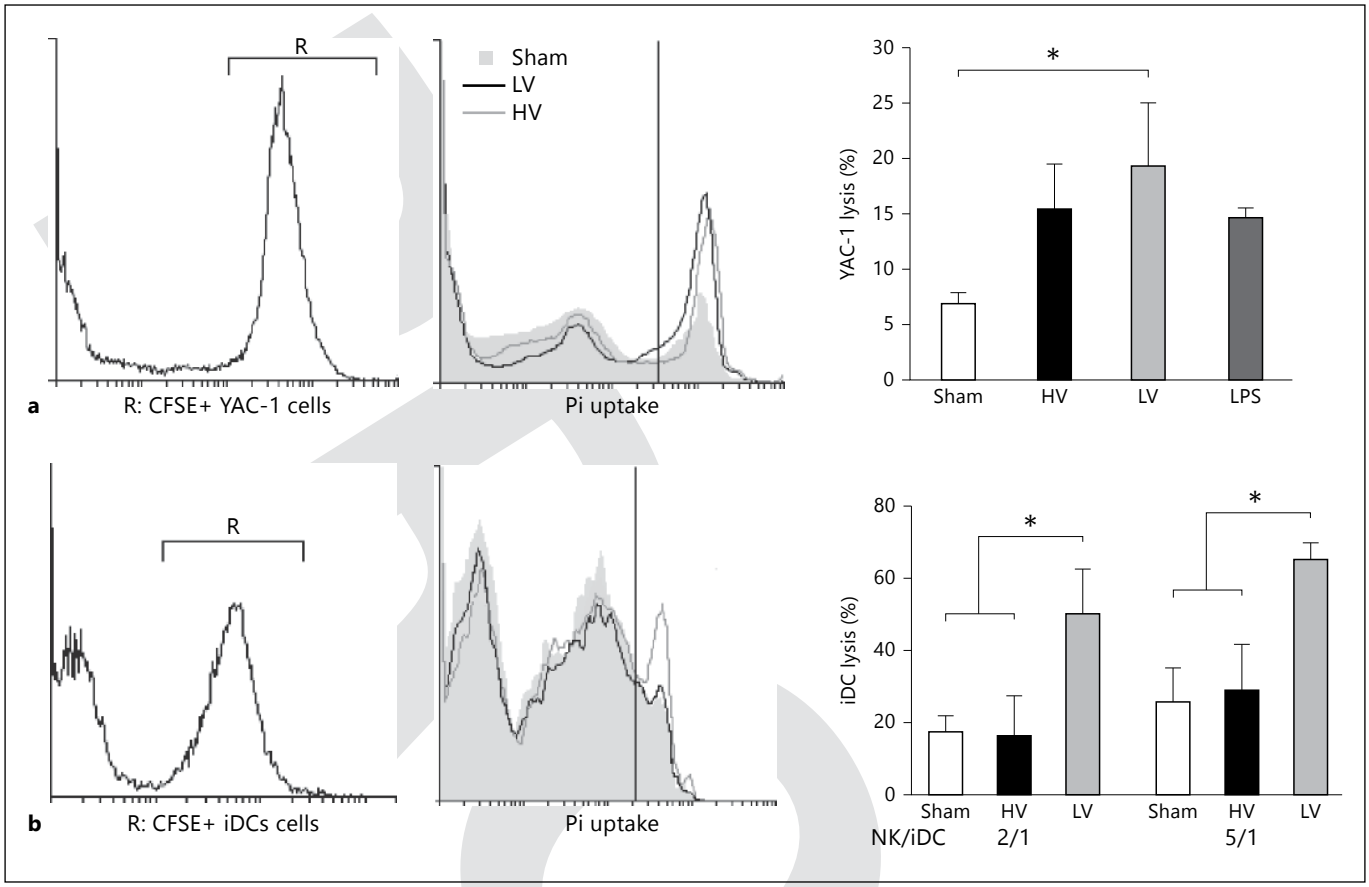

Fig. 4. NK cell-mediated lysis of YAC-1 cells and iDCs upon *T. cruzi* infection. **a** Lysis of YAC-1 cells by splenic NK cells from *T. cruzi* HV, LV or sham-infected (18 h.p.i.) and LPS (100 μg/mouse) inoculated C3H/HeN mice was evaluated. YAC-1 cells (5×10^4) were cultured with freshly purified splenic NK cells at a NK/YAC-1 ratio of 5/1. Left panel shows CFSE-labeled YAC-1 cells and middle panel shows PI uptake by CFSE+ cells gated in R. Results from a representative experiment with 3–5 pooled mice per group. Right panel show results of 3 independent experiments with 3–5 pooled mice per group (means ± SEM). ANOVA (Bonferroni's post hoc test). * $p < 0.05$ sham vs. LV and HV. **b** iDCs (5×10^4) were cultured with freshly purified spleen NK cells from HV, LV and sham-infected (18 h.p.i.) C3H/HeN mice. Left panel shows CFSE-labeled YAC-1 cells and middle panel shows PI uptake by CFSE+ cells gated in R. Results from a representative experiment with 3–5 pooled mice per group. Right panel shows results of 3 independent experiments with 3–5 pooled mice per group (means ± SEM). ANOVA (Bonferroni's post hoc test) * $p < 0.05$ sham vs. LV and HV.

(online suppl. fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000350242).

NK Cells from Mice Infected with T. cruzi HV Strain Display Reduced Capacity to Eliminate Autologous iDCs

First, we measured the cytotoxic function of NK cells isolated from the spleen of *T. cruzi*- and sham-infected mice using the NK-sensitive thymoma cell line YAC-1 as target. Infection with both *T. cruzi* populations (18 h post-infection, h.p.i.) raised the lytic capacity of splenic NK cells above sham-infected mice (fig. 4a). Mobilization of CD107a (LAMP-1) to the cell surface as a correlate of degranulation by activated circulating NK cells also increased significantly 18 h.p.i. with both HV and LV populations of *T. cruzi* (online suppl. fig. 2). NK cell-mediated elimination of iDCs contributes to the induction of immune responses via a reduction in the iDC pool. In view of the observed accumulation of DCs with poor maturation status after infection with the HV *T. cruzi* populations, we examined NK cell cytotoxicity of iDCs from infected animals. Therefore, we performed an in vitro cytotoxicity assay using iDCs derived from bone marrow precursors as targets. NK cells isolated from the spleen of mice infected with the LV *T. cruzi* strain increased significantly their rate of iDCs lysis compared to controls

(fig. 4b). In contrast, NK cells from mice infected with the HV strain failed to upregulate their capacity to lyse iDCs. Next, we tested the ability of NK cells from *T. cruzi*-infected mice to induce maturation/activation of iDCs. Coculture with NK cells from *T. cruzi*-infected mice failed to modulate the expression of MHC class II or to stimulate their cytokine secretion of TNF-α or IL-10 (online suppl. fig. 3).

IL-10 Participates in the NK Cell-Mediated Deletion of iDCs

Recently, it has been demonstrated that IL-10 affects NK cell function [29–31] and NK/DC crosstalk [32, 33]. As this regulatory cytokine is produced by different cell types upon infection with *T. cruzi* [34, 35], we tested whether IL-10 mediates NK cell-mediated deletion of iDCs. First, we cocultured NK cells from HV-infected mice with iDCs in the presence of an IL-10R blocking antibody (fig. 5a). The ability of NK cells to lyse iDCs was increased when IL-10 consumption was prevented by IL-10R blockade during NK-DC coculture. Next, NK cells from IL-10KO mice infected with the HV *T. cruzi* strain were cocultured with iDCs. The IL-10KO mice were of Balb/c background, a mouse strain susceptible to *T. cruzi* which also displays a divergent outcome upon infection with HV and LV *T. cruzi* populations [36]. NK cells obtained from IL-10KO infected mice displayed a significantly higher capacity to lyse iDCs (fig. 5b). Regarding DC maturation/activation, the absence of IL-10 during infection did not further confer the capacity to mediate in vitro maturation of DCs to NK cells (data not shown).

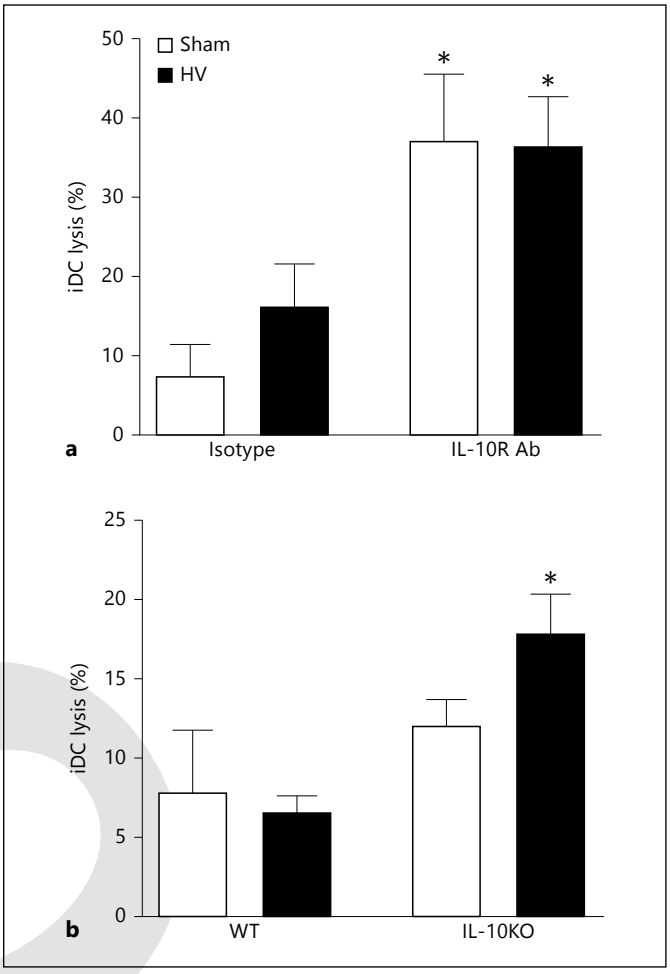

Fig. 5. IL-10 mediates NK cell cytotoxicity on iDCs. Mice were infected with HV *T. cruzi*. **a** iDCs (5×10^4 per well) were cultured with freshly purified spleen NK cells (NK/DC ratio: 2/1) from HV (■) and sham-infected (□) C3H/HeN male mice (18 h.p.i.) in the presence of an IL-10R blocking antibody (Ab; 40 μg/ml, clone 1B1.3A) or rat IgG isotype control (HPRN). Results are expressed as percent lysis from 2 independent experiments with 4–5 pooled mice per group (means ± SEM). ANOVA (Bonferroni's post hoc test). * $p < 0.05$ vs. sham isotype. **b** iDCs (5×10^4/well) were cultured with freshly purified spleen NK cells (NK/DC ratio: 2/1) from IL-10KO mice of Balb/c background or wild-type (WT) mice (18 h.p.i.). Results are expressed as percent lysis from 2 independent experiments with 4–5 pooled mice per group (means ± SEM). ANOVA (Bonferroni's post hoc test). * $p < 0.05$ vs. HV WT.

Discussion

Different studies show that NK cells mediate, at least in part, the clearance of iDCs [1, 2, 5, 6]. This mechanism optimizes the conditions for T-cell priming and confers to NK cells a role in shaping the response at the interphase of innate and adaptive immunity [7]. We have previously demonstrated that trypomastigotes and parasite-derived molecules from the HV strain downmodulate DC maturation and the capacity to activate T cells [17–19, 26]. Other authors have described a similar trend following infection with different HV *T. cruzi* strains [37, 38]. Here, the downregulation of splenic DC subset maturation is early noticeable (48 h.p.i.) and sustained during the acute phase of the infection with the HV strain but not with the LV strain. Moreover, the frequency, absolute number and MHC II expression of splenic DC subsets show that, during acute infection with the HV *T. cruzi* population, DCs with a poor maturation status accumulate in the spleen. The parasite burden in the spleen of mice infected with the HV strain increases notably from day 15 to 28 d.p.i. and correlates with the accumulation peak of iDCs in this secondary lymphoid organ.

Our results suggest that early in vivo *T. cruzi* infection does not stimulate the NK cell-induced capacity to mature or activate iDCs. Furthermore, splenic NK cells from mice infected with the HV *T. cruzi* strain but not with the LV strain are significantly impaired in their ability to eliminate autologous myeloid iDCs. This occurs even though NK cells from mice infected with both *T. cruzi* populations display similar cytotoxic capacity against YAC-1 cells. The discrepant sensitivity of cell targets to NK cell cytotoxicity is not unexpected since they seem to require different receptor-ligand interactions which are not fully characterized in mice [39]. In humans, NK-mediated killing of DCs appears to involve NKp30 [1] and the recognition on DCs of specific non-MHC ligands (i.e. costimulatory molecules) [4, 5]. Due to infection with the HV *T. cruzi* strain, NK cells probably lose their capacity to delete iDCs, which results in iDC accumulation in the spleen. Defective NK cell lysis of autologous iDCs was also demonstrated in the context of HIV infection [32, 40, 41]. Elimination of iDCs by NK cells from HIV-1-infected viremic patients is markedly reduced compared to that of NK cells from either aviremic HIV-1-infected subjects or healthy donors [41]. Aside from infectious diseases, NK cells from patients with acute myeloid leukemia display a defective ability to kill iDC [42]. The alteration in NK/DC cooperation was proposed by different authors as a common mechanism elicited by tumors and pathogens to escape from immune surveillance [7, 42]. Perhaps to avoid immune recognition, the intracellular protozoan *T. gondii* exploits direct contact of these cells in vitro via its transfer from infected DCs to effector NK cells [43].

IL-10 is a pleiotropic cytokine whose immunosuppressive impact has been well documented. In the course of *T. cruzi* infection, IL-10 is induced by various cell types, including diverse T-cell subsets and DCs [17, 18, 26, 34, 35]. We show here that blockade of IL-10R during NK/DC coculture or the absence of IL-10 during in vivo *T. cruzi* infection restores the capacity of NK cells to mediate killing of iDC. The IL-10-induced downmodulation of NK cell-mediated DC killing could also occur in vivo as the production of this cytokine by spleen cells is increased shortly after infection with the HV *T. cruzi* strain. The impact of this regulatory cytokine on NK-DC interactions during viral infection has been verified. IL-10 suppresses NK-DC crosstalk leading to poor priming of mouse cytomegalovirus-specific CD4 T cells and to viral persistence [33]. HIV induced IL-10 secretion makes DCs resistant to NK cell cytolysis [32]. In the steady state, IL-10 renders liver NK cells hyporesponsive, thus contributing to the tolerogenic environment of this organ [30].

Our data also demonstrate for the first time that early infection with the protozoan parasite *T. cruzi* increases significantly the proportion of circulating and splenic NK cells that produce IL-10. IL-10 production by NK cells has also been reported upon acute murine infection with rapidly disseminating bacteria, e.g. *Listeria monocytogenes, Yersinia pestis* and the parasitic protozoa *Leishmania donovani* [44] and *Toxoplasma gondii* [45]. It was demonstrated that during acute systemic, but not local infections, NK cells coproduce IFN-γ and IL-10 resulting in immune regulation [45]. Although we failed to detect the simultaneous production of both cytokines, we hypothesize that *T. cruzi*-induced IL-10 production by NK cells probably could lead to weakened host resistance or provide a negative feedback to limit inflammation. In fact, a regulatory role for NK cells during *T. cruzi* infection has been proposed by Cardillo et al. [46], who reported that NK1.1-positive cells are necessary at the beginning of infection to control susceptibility and T-cell hyperactivity of *T. cruzi*-infected mice.

The major role of NK cells shortly after *T. cruzi* infection is to contribute to trypanocidal mechanisms mainly through the early production of macrophage-activating cytokines as IFN-γ in response to infection [11, 12] or by directly killing extracellular parasites [14]. Accordingly, in our model, during the first hours of in vivo infection with HV and LV *T. cruzi* populations, increases in the frequency, CD69 expression and cytotoxic capacity of circulating NK cells were noted. We show here that *T. cruzi*-activated NK cells from the splenic compartment might also have an accessory role in regulating the strength of adaptive immune responses induced by DCs. Acute infection with the HV strain significantly impairs T-cell responses to mitogens, DC maturation/activation and, as we previously reported, stimulation of *T. cruzi*-specific CD4 T-cell responses probably delaying the induction of adaptive immune responses [19]. This parasite strain could also downmodulate in vivo the ability of NK cells to adjust the maturation level of the DC pool. Through this mechanism, probably mediated by IL-10, the parasite might engage NK cells and DCs in a regulatory circuit which leads to parasite persistence but prevents host death due to excessive inflammation. Understanding the interactions between DCs and NK cells that regulate anti-parasite responses at the frontier of innate and adaptive immunity could be relevant to design and improve strategies for pathogen control.

Acknowledgments

The authors thank Juan E. Ugalde (IIB-INTECH, UNSAM. CONICET) for kindly providing the IL-10$^{-/-}$ mice, Adriana Fontanal (Instituto Leloir, UBA-CONICET) for her assistance with mice and Maria Laura Belaunzarán for her aid with the figures. C.D.A.S., S.M.G.C. and A.G.S. are members of research career of CONICET. E.I.B. has a fellowship from the Facultad de Medicina, Universidad de Buenos Aires. C.V.P., T.D. and A.M.P.M. have fellowships from CONICET. Research was funded by grants from the Agencia Nacional de Promoción Cienctífica y Tecnológica (PICT 10-0866 to S.M.G.C.), the Fundación Bunge y Born to C.D.A.S., UBACyT (M601 and M066 to C.D.A.S.) and CONICET (PIP 112-200801-00769 to S.M.G.C. and PIP 112-20081-02915 to A.G.S.).

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