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Accessory cell dependent NK cell mediated PBMC IFN- γ production is defective in HIV infection

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Abstract

HCV and HIV infections impair dendritic cell function. We evaluated the impact of HCV, HIV, and HCV-HIV infection on MDC–NK interactions by analyzing CD3 depleted PBMC for NK cell IFN- γ in response to IL-12 or poly (I:C). Purified MDC and NK cells were analyzed for TLR ligand-dependent, MDC-dependent NK activity. In HIV infection, IFN- γ production by CD3 depleted PBMC was reduced in response to poly (I:C), while response to IL-12 was intact in HCV and HIV infections. Poly (I:C) induced activity was dependent on MDC and partially dependent on IL-12, consistent with accessory cell help. In purified MDC–NK co-cultures, MDC dependent NK IFN- γ and Granzyme B was intact in both HCV and HIV infections, while MDC numerical defects were observed in HIV infection. These data indicate that during viral infection with HIV, accessory cell dependent NK function in the periphery is impaired. This impairment may be related to the identified MDC numerical defect.

Keywords

Human; Natural killer cell; IL-12; Dendritic cell; IFN-gamma; HIV; HCV

Introduction

Hepatitis C virus (*HCV*) is the most common cause of chronic viral hepatitis in the United States [1]. HIV infection appears to alter the course of HCV related disease, accelerating rates of progression to cirrhosis [2]. Despite a high prevalence of HCV-HIV coinfection, the interrelationships between these viruses are not well understood, though immune mediated mechanisms of interaction are likely. Host innate immune response to viral infection is thought to rely in part on immature dendritic cells (*DC*). *MDC and PDC*, or myeloid and

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plasmacytoid DC, have emerged as two main peripheral DC subsets [3]. Numerical and functional defects in these populations have been described in both HCV and HIV infections, with the impairment quite different in each infection [4–12].

Natural killer (NK) cells are an innate immune cell population of T cell receptor negative (CD3⁻) lymphocytes that are capable of cytotoxic, cytokine expressing, and chemokine expressing functions [13,14]. These lymphocytes have been well established as a vital early innate cellular defense during mouse hepatic viral infection [15–19], and in human herpes virus infection [20–26]. In the setting of HIV infection, HIV exposed but uninfected subjects have been reported to have enhanced NK function [27]. In contrast, during chronic HIV infection, NK cytotoxic function has long been known to be reduced in subjects with AIDS [28,29], and genetically determined NK phenotype is associated with disease progression rate [30]. After HAART induced immune restoration, NK numbers and function (IFN- γ production) appear to normalize [31,32]. In the setting of HCV infection, genetically determined NK phenotype appears to affect the course of HCV infection after acute exposure [33]. During chronic HCV infection, studies of NK function have yielded mixed results, with some studies indicating reduced NK cytotoxicity in the periphery of HCV infected subjects [34–36], and others indicating normal NK function [37–39]. Most studies indicate reduced NK cell numbers in the setting of chronic HCV infection [34,35,37,39]. NK function and numbers have not been well studied in HCV-HIV coinfection, with one report indicating reduced NK numbers and cytotoxicity [34].

DC–NK bi-directional cross-talk has been described as playing an important role in host immunity [40–42]. Cell contact and/or cytokine mediated communication between DC and NK cells results in NK activation, enhanced NK effector function, and increased DC maturation [42,43]. Furthermore, in the mouse system where DC–NK interactions are found to be necessary for optimal control of pathogens, the cell–cell interaction has been found to occur in the lymph node, in similar fashion to classically described DC–T interactions [44]. Therefore, DC–NK bidirectional interactions may provide an efficient means of generating an effective innate immune response, which in turn could act as a bridge to an effective adaptive immune response.

In vitro monocyte derived MDC are capable of activating NK cells during early stages of HIV infection, with activity inversely relating to plasma HIV level [45]. In chronic HIV infection, this activity is diminished [45,46]. In HCV infection, *in vitro* monocyte derived MDC–NK interactions have also been described as impaired [47,48]. However, whether such defects are present *in vivo*, and whether similar or other defects are present in direct *ex vivo* assays using freshly prepared MDC is unclear. Moreover, the impact of HCV-HIV coinfection on these activities is not known.

In this study, we evaluated accessory cell dependent NK cell activation in un-fractionated, freshly prepared peripheral blood cell populations from HCV, HIV, HCV-HIV and healthy control subjects. In CD3 depleted PBMC from HIV infected subjects, we observed a defect in poly (I:C) induced IFN- γ . This activity is shown to be associated with, and partially dependent upon IL-12, which is likely secreted by the MDC fraction also found to be required for this assay activity. Further characterization of this defect was performed by

isolation of NK and MDC from HIV, HCV and healthy control subjects. Upon cell isolation, reduced yields of MDC were observed in the HIV infected group. However, MDC dependent NK IFN- γ and Granzyme B in the presence of poly (I:C) stimulation was found to be intact in both HCV and HIV infections. These data suggest that numerical defects in peripherally circulating MDC in HIV infection may be responsible for the defect observed in un-fractionated direct *ex-vivo* cell assays.

Methods

Study subject identification and IRB approval

Chronic HCV infected subjects ($n=29$) had detectable serum HCV antibody for over 6 months duration, had detectable HCV RNA by PCR, and were not previously treated for HCV infection. HCV-HIV coinfecting subjects ($n=9$), in addition, had detectable HIV antibodies. All HIV infected subjects ($n=30$) had detectable HIV antibodies by both ELISA and Western blot, while healthy control subjects ($n=25$) were negative for both HCV and HIV antibodies. All study subjects provided written informed consent, conforming to the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000, under approval of the institutional review boards for human studies at the Cleveland VA Medical Center and University Hospitals of Cleveland.

Flow cytometric analysis

Flow cytometric analysis was performed on a FACScalibur (Becton Dickinson, San Jose, CA) flow cytometer with Cell Quest Software (Becton Dickinson) using freshly prepared peripheral blood mononuclear cells (PBMC; 1×10^6 PBMC analyzed per sample). PBMC were stained with anti-CD3, and anti-CD56 mAb (Becton Dickinson, San Jose, CA) for 20 min at 25 °C then washed in PBS with 0.01% BSA. Lymphocytes were identified by forward and side scatter, and the dominant NK cell subset frequency was determined as the proportion of live lymphocyte gated cells that expressed CD56, and did not express CD3. For analysis of IFN- γ production by flow cytometry, cells were cultured in the presence of the appropriate stimulus in the presence of brefeldin A, then permeabilized and stained with anti-IFN- γ mAb (Becton Dickinson, San Jose, CA) for 30 min at 25 °C, then washed in PBS with 0.01% BSA and fixed in 1% paraformaldehyde, then stored at 4 °C until analysis.

IFN- γ and Granzyme B ELISPOT assays

CD3 depleted PBMC (3×10^5 cells/well, StemCell Technologies, Vancouver, BC) were plated on to ninety-six well IFN- γ ELISPOT plates in duplicate, in the presence or absence of recombinant IL-12 (1 ng/ml, R&D Systems, Minneapolis, MN) or poly (I:C) (50 μ g/ml, Amersham Biosciences, Piscataway, NJ). Cells were incubated for 20 h at 37 °C, and IFN- γ ELISPOT assays were developed and analyzed as previously described [49]. For blocking experiments, IL-12 neutralizing Ab (10 μ g/ml, Becton Dickinson, San Jose, CA) or the isotype control Ab (10 μ g/ml, Becton Dickinson, San Jose, CA) was added in duplicate. For MDC depletion experiments BDCA-1 bearing cells were depleted by bead method (Miltenyi Biotech, Auburn, CA).

For purified cell assays, MDC were prepared using the BDCA1 isolation kit (Miltenyi Biotech, Auburn, CA), by first removing CD19 expressing cells, then positively selecting BDCA-1 expressing cells. NK cells were prepared by a negative selection method, first removing CD19, BDCA1, and BDCA2 expressing cells (Miltenyi Biotech, Auburn, CA), followed by removing CD3, CD4, and CD14 expressing cells (EasySep, StemCell Technologies, Vancouver, BC). Freshly isolated NK cells (1×10^5 cells/well, unless otherwise stated) were plated in the presence or absence of freshly isolated autologous MDC (2×10^4 cells/well). Initial assays performed using healthy control cell populations indicated that this ratio of MDC to NK cells was in a linear range for MDC enhancement of NK IFN- γ producing function. The cells were plated in ninety-six well IFN- γ or Granzyme B ELISPOT plates and were cultured in the presence or absence of poly (I:C) and/or K562 target cells (2×10^4 cells/well, ATCC, Manassas, VA) for 20 h at 37 °C, then developed and analyzed as described above for IFN- γ , and as per kit instruction for Granzyme B (Becton Dickinson, San Jose, CA).

IL-12 ELISA

Freshly isolated PBMC (6×10^5 cells/well), NK cells (2×10^5 cells/well), monocytes (2×10^5 cells/well, EasySep positive CD14 bead selection, Stem Cell Technologies, Vancouver, BC) or dendritic cells (5×10^4 cells/well, dendritic cell isolation kit, Miltenyi Biotech, Auburn, CA) were suspended in RPMI containing 5% human serum, 1% L-glutamine, 1% penicillin/streptomycin and plated in ninety-six well tissue culture plates in the presence or absence of poly (I:C) (50 μ g/ml). Cell cultures were incubated for 20 h at 37 °C, supernatants were removed and IL-12 production was measured by ELISA (R&D Systems, Minneapolis, MN).

Statistical analysis

We analyzed the data with SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL) and Stata SE, v. 9.2, (Stata Corp., College Station, TX). We used conventional measures of central tendency and variability to describe the data. To compare continuous and categorical variables across groups, we used Mann–Whitney’s *U* test or Kruskal–Wallis’ *H* test and Pearson’s chi-square, respectively. We compared responses before and after stimulation, and those with and without target cells with Wilcoxon’s signed rank test, and assessed the associations between continuous variables by Spearman’s rank correlation coefficient. All tests were two-sided, and a *p*-value ≤ 0.05 was considered significant.

Results

Poly (I:C) induced CD3 depleted PBMC IFN- γ is impaired in HIV and HCV-HIV infections, while IL-12 induced activity is intact

NK cell responses are thought to be triggered by a variety of mechanisms including direct and accessory cell mediated activation [50]. In order to investigate direct and accessory cell mediated NK function, we utilized reagents known to activate NK and DC populations. While IL-12 directly activates NK cells, poly (I:C) activates both MDC and NK cells, and is known to facilitate MDC–NK interactions [42,43]. As expected, in our system CD3-/56+ NK cell IFN- γ can be induced by stimulating PBMC with the TLR3 ligand poly (I:C) or IL-12 (Fig. 1A).

To best approximate *in-vivo* NK and accessory cell mediated NK function, we first analyzed unfractionated PBMC samples freshly prepared from 15 chronic HCV infected (without prior therapy for HCV), 15 HIV infected, 9 HCV-HIV infected, and 15 healthy control subjects. Clinical characteristics are shown in Table 1. 11 of 15 HIV infected and 8 of 9 HCV-HIV infected subjects were receiving antiretroviral therapy. CD4 nadir ranged from 60 to 473 in HIV infected subjects and 50–346 in HCV-HIV infected subjects. Flow cytometric analysis of PBMC for frequencies of CD3-CD56+ NK cells revealed a numerical defect in this NK population in HIV and HCV-HIV infected subjects when compared to controls (median NK frequency 7.75%, 6.42%, 5.32%, and 10.94% for HCV, HIV, HCV-HIV, and control subjects; $p=0.15$ HCV vs. control, $p=0.004$ HIV vs. control, and $p=0.009$ HCV-HIV vs. control), a finding that is consistent with previous reports of diminished numbers of circulating CD3-56+ NK cell frequencies during HCV and HIV infections [32,39,51]. Notably, only one NK subset is considered in this flow based analysis, while other NK subsets have been shown to be altered in distribution during HIV infection [52]. We next examined CD3 depleted PBMC for NK cell IFN- γ production in response to poly (I:C) and IL-12 stimulation. This cell fraction contains all possible NK subsets. CD3 depletion was performed to avoid measurement of T cell IFN- γ . There was a broad range of responses to poly (I:C) stimulation within each group, and responses were lower in HIV infected and HCV-HIV infected subjects, but not in chronic HCV infected subjects when compared to healthy controls (median for HCV, HIV, HCV-HIV, and control subjects 75, 30, 7, and 198 SFU/300,000 CD3 depleted PBMC; $p=0.2$ HCV vs. control, $p=0.005$ HIV vs. control, and $p=0.03$ HCV-HIV vs. control) (Fig. 1B). In contrast, all groups had comparable IFN- γ production in response to IL-12 (Fig. 1C), indicating that one component of NK function upon direct stimulation is intact during HIV infection.

Since our observed defect in poly (I:C) induced activity may be related to a defect in accessory cell activity, and because monocyte derived MDC dependent NK IFN- γ is known to be cell contact and IL-12 dependent [43], we next evaluated whether poly (I:C) induced CD3-PBMC IFN- γ is associated with IL-12 production. As shown in Fig. 1D this was found to be the case when data from all subjects were combined, and the correlation coefficients for each group were similar to the coefficient with all groups combined.

There was no observed correlation between any of the clinical variables (HCV level, HIV level, ALT, or CD4 cell count) and functional activities measured.

IFN- γ production by CD3 depleted PBMC in response to poly (I:C) is dependent on IL-12 and the presence of MDC

To further explore the dependence of our unfractionated system on accessory cell function, we evaluated whether the poly (I:C) induced activity was IL-12 dependent. In control subjects, IL-12 blocking assays revealed this activity was partially dependent on IL-12 (Fig. 2A). To evaluate the role of MDC in this system, we performed assays in the presence or absence of MDC. As shown in Fig. 2B, IFN- γ production by CD3-PBMC in response to poly (I:C) stimulation was dependent on the presence of MDC. To determine which PBMC cell population is capable of secreting IL-12 in response to poly (I:C), we next performed assays on enriched cell fractions (Fig. 2C). The DC fraction was most capable of IL-12

production in these assays, indicating that this is a likely source of IL-12 in the unfractionated assays.

MDC numbers are reduced in HIV infection while in purified cell assays MDC dependent NK IFN- γ production is intact

Since the defect observed in the unfractionated system may be due to reduced MDC numbers, reduced MDC function, or reduced NK cell responsiveness, we next evaluated purified MDC and NK cells for TLR ligand dependent activation. To avoid possible *in vitro* culture artifact that may be observed with monocyte derived MDC, we focused here on freshly isolated MDC. We isolated MDC and NK cell fractions from 9 healthy controls, 8 HIV infected, and 10 HCV infected subjects. For these assays all HIV-infected subjects were viremic and had been off antiretroviral treatment for at least 3 months. Median plasma HIV RNA level was 15,200 copies/ ml, and median CD4 count was 490 cells/mm³. HCV-infected subjects were all treatment naïve and median plasma HCV level was 1,308,500 IU/ml.

As shown in Fig. 3, reduced MDC numbers were obtained from the HIV infected subjects, consistent with previous reports of reduced circulating MDC cell numbers during HIV infection [4,6]. Although reduced MDC numbers have been found previously by others during HCV infection[7,10,12] MDC yield in HCV infected subject samples were not different from those of controls.

To measure MDC dependence of NK IFN- γ in the setting of poly (I:C) stimulation, NK cells were stimulated with poly (I:C) in the presence and absence of autologous MDC. In the absence of MDC, NK cells purified from healthy control subjects produce little IFN- γ in response to poly (I:C), while the same cells respond to IL-12 stimulation (Fig. 4A). Because the presence of K562 target cells facilitate NK cell activation and IFN- γ production [53], we also performed MDC–NK assays in the presence or absence of target cells. As shown in Fig. 4B for a representative healthy control sample, the presence of MDC enhances poly (I:C) induced NK IFN- γ and this activity is further enhanced by the presence of K562 target cells. When analyzing activities in our groups of subjects poly (I:C) induced NK IFN- γ in the absence of MDC was similar among HIV, HCV, and healthy control groups (median 4 vs. 19 vs. 6 SFU/well, $p>0.5$, *not shown*). Furthermore, MDC dependent enhancement of NK IFN- γ in response to poly (I:C) was found to be intact in both HIV and HCV infections as compared to controls (median 8, 15, and 14 SFU/well, $p>0.5$, Fig. 4C). This was also the case in the presence of K562 target cells (median 26, 68, and 31 SFU/well $p>0.6$ Fig. 4D). To investigate a separate NK activity, we measured MDC dependent NK Granzyme B secreting cell frequency. This activity was also enhanced by the presence of K562 target cells (*not shown*). Similar to the case with IFN- γ secreting activity, MDC dependent NK Granzyme B secreting activity in the presence of K562 target cells was similar comparing HIV, HCV, and healthy control samples (15, 18, and 26 SFU/well $p>0.6$ Fig. 4E).

Discussion

MDC–NK interactions may facilitate an effective HIV or HCV directed immune response. Here we evaluated freshly prepared unfractionated and fractionated cells in direct *ex-vivo*

assays for TLR ligand dependent, accessory cell mediated NK activity. In the unfractionated system, we identified a defect in poly (I:C), but not IL-12 induced IFN- γ production by CD3 depleted PBMC in HIV infected subjects. Because this activity was associated with IL-12 production, in part dependent on IL-12, and dependent on the presence of MDC we conclude that this activity is at least in part accessory cell mediated. To investigate this accessory cell dependent NK defect in HIV infection, we then performed direct *ex-vivo* assays with freshly purified MDC and NK cells. An MDC numerical defect was identified in HIV infected subjects, while on a per cell basis poly (I:C) induced, MDC dependent NK activity was found to be intact in HIV and HCV infected subjects. These latter data suggest an MDC numerical defect may contribute to the reduced accessory cell mediated NK activity in our unfractionated system. Importantly, in addition to MDC numerical defects, CD56 expressing NK numerical defects were also identified in the unfractionated system. However, we found intact CD3 depleted PBMC IFN- γ production in response to IL-12, suggesting that the NK subset numerical defect does not fully explain the defect in response to poly (I:C).

The role of IL-12 in the MDC–NK interaction has been established [43]. Here we find poly (I:C) dependent CD3 depleted PBMC IFN- γ is associated with, and partially dependent on, IL-12, consistent with IL-12 playing a role in the MDC mediated accessory cell dependent NK function. Though previous reports have described a defect in TLR ligand induced PBMC IL-12 during HIV infection [4,54,55], we did not find such a defect in the unfractionated assays performed here (*not shown*), indicating that the activity defect in the unfractionated system is not solely due to a defect in accessory cell IL-12 production. In fact, in the purified cell assay supernatants IL-12 was not detected (*not shown*). While the latter finding does not exclude a role for IL-12 in the MDC–NK interaction, it does suggest that any defect in MDC IL-12 production that exists in the setting of HIV infection does not appear to affect the intact poly (I:C) induced MDC–NK activity observed here.

In HIV infection, MDC numbers have been previously reported as decreased [5,6], and MDC are well known to activate NK cells by mechanisms that include soluble factors such as IL-12 and cell surface receptors such as NKP30 [42,43,46]. Here we identify an accessory cell mediated NK defect in HIV infection that appears to be related to MDC numerical defects, while on a per cell basis MDC function was found to be intact. In contrast to our findings here, previous studies of monocyte derived MDC obtained from HCV and HIV infected subjects have indicated impaired activity on a per cell basis [45–48]. These differing results may be due to differences between *in vitro* derived monocyte derived MDC used in other studies and the freshly prepared MDC used here. In support of this possibility, differences in maturation and cytokine secreting function comparing MDC and *in vitro* derived monocyte derived MDC have been previously described [56].

The potential clinical implications of our findings are unknown, but may include effects on immune control of HIV replication, maintenance of immunity against opportunistic infections, and susceptibility to malignant diseases. NK cell phenotype or function appears to be enhanced in slow progressors and in highly exposed seronegative individuals, highlighting the potential role of NK cell function on HIV control [27,30]. NK cells are also an important source of the β -chemokines MIP-1 α , MIP-1 β and RANTES [13,14], which are

potent inhibitors of HIV replication. We did not have access to longitudinally obtained samples in our subjects, and therefore cannot assess how the defects we found relate to disease progression, but they may play a more prominent role in late disease, a population that was not represented in our study sample. NK cell function may also play a role in response against CMV infection and other viral pathogens, and NK cells are one of the most potent effectors active against neoplastic cells. Moreover, MDC–NK interactions may play an important role in lymph node localized HIV pathogenesis, and our studies of peripherally localized cells cannot identify whether there may be a lymph node localized defect in this interaction. Thus, progressive defects in accessory cell dependent NK function might contribute to the emergence of non-AIDS-defining malignancies, an emerging cause of morbidity and mortality in HIV-infected patients.

In conclusion, impaired accessory cell dependent NK function is present in HIV infection, though this defect is not directly attributable to diminished MDC or NK function. We favor a numerical defect in MDC as explaining this observation.

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Abbreviations

ALT	alanine aminotransferase
DC	dendritic cell
ELISPOT	enzyme linked immunospot assay
HCV	hepatitis C virus
HAART	highly active anti-retroviral therapy
IFN-γ	interferon gamma
IL	interleukin
MDC	myeloid dendritic cell
PBMC	peripheral blood mononuclear cell
PDC	plasmacytoid dendritic cell
HIV	human immunodeficiency virus
NK	natural killer
SFU	spot forming units
TLR	toll like receptor

References

1. Lauer GM, Walker BD. Hepatitis C virus infection. *N. Engl. J. Med.* 2001; 345:41–52. [PubMed: 11439948]

2. Cacoub P, Geffray L, Rosenthal E, Perronne C, Veyssier P, Raguin G. Mortality among human immunodeficiency virus-infected patients with cirrhosis or hepatocellular carcinoma due to hepatitis C virus in French Departments of Internal Medicine/Infectious Diseases, in 1995 and 1997. *Clin. Infect. Dis.* 2001; 32:1207–1214. [PubMed: 11283811]
3. Rissoan MC, Soumelis V, Kadowaki N, Grouard G, Briere F, de Waal Malefyt R, Liu YJ. Reciprocal control of T helper cell and dendritic cell differentiation. *Science.* 283(1999):1183–1186. [PubMed: 10024247]
4. Anthony DD, Yonkers NL, Post AB, Asaad R, Heinzel FP, Lederman MM, Lehmann PV, Valdez H. Selective impairments in dendritic cell associated function distinguish HCV and HIV infection. *J. Immunol.* 2004; 172:4907–4916. [PubMed: 15067070]
5. Chehimi J, Campbell DE, Azzoni L, Bacheller D, Papasavvas E, Jerandi G, Mounzer K, Kostman J, Trinchieri G, Montaner LJ. Persistent decreases in blood plasmacytoid dendritic cell number and function despite effective highly active antiretroviral therapy and increased blood myeloid dendritic cells in HIV-infected individuals. *J. Immunol.* 2002; 168:4796–4801. [PubMed: 11971031]
6. Feldman S, Stein D, Amrute S, Denny T, Garcia Z, Kloser P, Sun Y, Megjugorac N, Fitzgerald-Bocarsly P. Decreased interferon-alpha production in HIV-infected patients correlates with numerical and functional deficiencies in circulating type 2 dendritic cell precursors. *Clin. Immunol.* 2001; 101:201–210. [PubMed: 11683579]
7. Kanto T, Inoue M, Miyatake H, Sato A, Sakakibara M, Yakushijin T, Oki C, Itose I, Hiramatsu N, Takehara T, Kasahara A, Hayashi N. Reduced numbers and impaired ability of myeloid and plasmacytoid dendritic cells to polarize T helper cells in chronic hepatitis C virus infection. *J. Infect. Dis.* 2004; 190:1919–1926. [PubMed: 15529255]
8. Soumelis V, Scott I, Gheys F, Bouhour D, Cozon G, Cotte L, Huang L, Levy JA, Liu YJ. Depletion of circulating natural type 1 interferon-producing cells in HIV-infected AIDS patients. *Blood.* 2001; 98:906–912. [PubMed: 11493432]
9. Ulsenheimer A, Gerlach JT, Jung MC, Gruener N, Wachtler M, Backmund M, Santantonio T, Schraut W, Heeg MH, Schirren CA, Zachoval R, Pape GR, Diepolder HM. Plasmacytoid dendritic cells in acute and chronic hepatitis C virus infection. *Hepatology.* 2005; 41:643–651. [PubMed: 15726647]
10. Wertheimer AM, Bakke A, Rosen HR. Direct enumeration and functional assessment of circulating dendritic cells in patients with liver disease. *Hepatology.* 2004; 40:335–345. [PubMed: 15368438]
11. Yonkers NL, Rodriguez B, Milkovich KA, Asaad R, Lederman MM, Heeger PS, Anthony DD. TLR ligand-dependent activation of naive CD4 T cells by plasmacytoid dendritic cells is impaired in hepatitis C virus infection. *J. Immunol.* 2007; 178:4436–4444. [PubMed: 17372001]
12. Longman RS, Talal AH, Jacobson IM, Rice CM, Albert ML. Normal functional capacity in circulating myeloid and plasmacytoid dendritic cells in patients with chronic hepatitis C. *J. Infect. Dis.* 2005; 192:497–503. [PubMed: 15995965]
13. Moretta L, Ciccone E, Poggi A, Mingari MC, Moretta A. Ontogeny specific functions and receptors of human natural killer cells. *Immunol. Lett.* 1994; 40:83–88. [PubMed: 8088876]
14. Lanier LL, Spits H, Phillips JH. The developmental relationship between NK cells and T cells. *Immunol. Today.* 1992; 13:392–395. [PubMed: 1418374]
15. Kakimi K, Guidotti LG, Koezuka Y, Chisari FV. Natural killer T cell activation inhibits hepatitis B virus replication In vivo [In Process Citation]. *J. Exp. Med.* 2000; 192:921–930. [PubMed: 11015434]
16. Liu ZX, Govindarajan S, Okamoto S, Dennert G. NK cells cause liver injury and facilitate the induction of T cell-mediated immunity to a viral liver infection. *J. Immunol.* 2000; 164:6480–6486. [PubMed: 10843705]
17. Habu S, Akamatsu K, Tamaoki N, Okumura K. In vivo significance of NK cell on resistance against virus (HSV-1) infections in mice. *J. Immunol.* 1984; 133:2743–2747. [PubMed: 6207244]
18. McIntyre KW, Welsh RM. Accumulation of natural killer and cytotoxic T large granular lymphocytes in the liver during virus infection. *J. Exp. Med.* 1986; 164:1667–1681. [PubMed: 3490535]

19. Rager-Zisman B, Quan PC, Rosner M, Moller JR, Bloom BR. Role of NK cells in protection of mice against herpes simplex virus-1 infection. *J. Immunol.* 1987; 138:884–888. [PubMed: 3805719]
20. Biron CA, Byron KS, Sullivan JL. Severe herpesvirus infections in an adolescent without natural killer cells [see comments]. *N. Engl. J. Med.* 1989; 320:1731–1735. [PubMed: 2543925]
21. Joncas J, Monczak Y, Ghibu F, Alfieri C, Bonin A, Ahronheim G, Rivard G. Brief report: killer cell defect and persistent immunological abnormalities in two patients with chronic active Epstein-Barr virus infection. *J. Med. Virol.* 1989; 28:110–117. [PubMed: 2544675]
22. Ballas ZK, Turner JM, Turner DA, Goetzman EA, Kemp JD. A patient with simultaneous absence of “classical” natural killer cells (CD3–, CD16+, and NKH1+) and expansion of CD3+, CD4–, CD8–, NKH1+ subset. *J. Allergy Clin. Immunol.* 1990; 85:453–459. [PubMed: 2303649]
23. Goodyear HM, McLeish P, Randall S, Buchan A, Skinner GR, Winther M, Rolland J, Morgan G, Harper JJ. Immunological studies of herpes simplex virus infection in children with atopic eczema. *Br. J. Dermatol.* 1996; 134:85–93. [PubMed: 8745891]
24. Cauda R, Laghi V, Tumbarello M, Ortona L, Whitley RJ. Immunological alterations associated with recurrent herpes simplex genitalis. *Clin. Immunol. Immunopathol.* 1989; 51:294–302. [PubMed: 2539283]
25. Quinnan GV, Kirmani N, Rook AH, Manischewitz JF, Jackson L, Moreschi G, Santos GW, Saral R, Burns WH. Cytotoxic t cells in cytomegalovirus infection: HLA-restricted T-lymphocyte and non-T-lymphocyte cytotoxic responses correlate with recovery from cytomegalovirus infection in bone-marrow-transplant recipients. *N. Engl. J. Med.* 1982; 307:7–13. [PubMed: 6281647]
26. Yamamoto T, Osaki T, Yoneda K, Ueta E. Immunological investigation of adult patients with primary herpes simplex virus-1 infection. *J. Oral Pathol. Med.* 1993; 22:263–267. [PubMed: 8102649]
27. Scott-Algara D, Truong LX, Versmisse P, David A, Luong TT, Nguyen NV, Theodorou I, Barre-Sinoussi F, Pancino G. Cutting edge: increased NK cell activity in HIV-1-exposed but uninfected Vietnamese intravascular drug users. *J. Immunol.* 2003; 171:5663–5667. [PubMed: 14634071]
28. Rook AH, Masur H, Lane HC, Frederick W, Kasahara T, Macher AM, Djeu JY, Manischewitz JF, Jackson L, Fauci AS, Quinnan GV Jr. Interleukin-2 enhances the depressed natural killer and cytomegalovirus-specific cytotoxic activities of lymphocytes from patients with the acquired immune deficiency syndrome. *J. Clin. Invest.* 1983; 72:398–403. [PubMed: 6308051]
29. Lederman MM, Ratnoff OD, Scillian JJ, Jones PK, Schacter B. Impaired cell-mediated immunity in patients with classic hemophilia. *N. Engl. J. Med.* 1983; 308:79–83. [PubMed: 6216408]
30. Martin MP, Gao X, Lee JH, Nelson GW, Detels R, Goedert JJ, Buchbinder S, Hoots K, Vlahov D, Trowsdale J, Wilson M, S J, Brien O, Carrington M. Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. *Nat. Genet.* 2002; 31:429–434. [PubMed: 12134147]
31. Azzoni L, Papasavvas E, Chehimi J, Kostman JR, Mounzer K, Ondercin J, Perussia B, Montaner LJ. Sustained impairment of IFN-gamma secretion in suppressed HIV-infected patients despite mature NK cell recovery: evidence for a defective reconstitution of innate immunity. *J. Immunol.* 2002; 168:5764–5770. [PubMed: 12023377]
32. Alter G, Malenfant JM, Delabre RM, Burgett NC, Yu XG, Lichterfeld M, Zaunders J, Altfeld M. Increased natural killer cell activity in viremic HIV-1 infection. *J. Immunol.* 2004; 173:5305–5311. [PubMed: 15470077]
33. Khakoo SI, Thio CL, Martin MP, Brooks CR, Gao X, Astemborski J, Cheng J, Goedert JJ, Vlahov D, Hilgartner M, Cox S, Little AM, Alexander GJ, Cramp ME, S J, Brien O, Rosenberg WM, Thomas DL, Carrington M. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science.* 2004; 305:872–874. [PubMed: 15297676]
34. Voiculescu CL, Balasoiu M, Turculeanu A, Radu C, Avramescu C, Radu E. Different patterns of some systemic immunological cell markers in HIV only, and HIV/hepatitis C-infected children. *Pediatr. AIDS HIV Infect.* 1996; 7:31–36.
35. Par G, Rukavina D, Podack E, Horanyi M, Szekeres-Bartho J, Hegedus G, Paal M, Szereday L, Mozsik G, Par A. Decrease in CD3-negative-CD8dim(+) and Vdelta2/Vgamma9 TcR+ peripheral blood lymphocyte counts, low perforin expression and the impairment of natural killer cell activity

- is associated with chronic hepatitis C virus infection. *J. Hepatol.* 2002; 37:514. [PubMed: 12217606]
36. Corado J, Toro F, Rivera H, Bianco NE, Deibis L, De Sanctis JB. Impairment of natural killer (NK) cytotoxic activity in hepatitis C virus (HCV) infection. *Clin. Exp. Immunol.* 1997; 109:451–457. [PubMed: 9328121]
 37. Kawarabayashi N, Seki S, Hatsuse K, Ohkawa T, Koike Y, Aihara T, Habu Y, Nakagawa R, Ami K, Hiraide H, Mochizuki H. Decrease of CD56(+)T cells and natural killer cells in cirrhotic livers with hepatitis C may be involved in their susceptibility to hepatocellular carcinoma. *Hepatology.* 2000; 32:962–969. [PubMed: 11050046]
 38. Duesberg U, Schneiders AM, Flieger D, Inchauspe G, Sauerbruch T, Spengler U. Natural cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC) is not impaired in patients suffering from chronic hepatitis C. *J. Hepatol.* 2001; 35:650–657. [PubMed: 11690712]
 39. Morishima C, Paschal DM, Wang CC, Yoshihara CS, Wood BL, Yeo AE, Emerson SS, Shuhart MC, Gretch DR. Decreased NK cell frequency in chronic hepatitis C does not affect ex vivo cytolytic killing. *Hepatology.* 2006; 43:573–580. [PubMed: 16496327]
 40. Mailliard RB, Son YI, Redlinger R, Coates PT, Giermasz A, Morel PA, Storkus WJ, Kalinski P. Dendritic cells mediate NK cell help for Th1 and CTL responses: two-signal requirement for the induction of NK cell helper function. *J. Immunol.* 2003; 171:2366–2373. [PubMed: 12928383]
 41. Mocikat R, Braumuller H, Gumy A, Egeter O, Ziegler H, Reusch U, Bubeck A, Louis J, Mailhammer R, Riethmuller G, Koszinowski U, Rocken M. Natural killer cells activated by MHC class I(low) targets prime dendritic cells to induce protective CD8 T cell responses. *Immunity.* 2003; 19:561–569. [PubMed: 14563320]
 42. Gerosa F, Baldani-Guerra B, Nisii C, Marchesini V, Carra G, Trinchieri G. Reciprocal activating interaction between natural killer cells and dendritic cells. *J. Exp. Med.* 2002; 195:327–333. [PubMed: 11828007]
 43. Gerosa F, Gobbi A, Zorzi P, Burg S, Briere F, Carra G, Trinchieri G. The reciprocal interaction of NK cells with plasma-cytoid or myeloid dendritic cells profoundly affects innate resistance functions. *J. Immunol.* 2005; 174:727–734. [PubMed: 15634892]
 44. Lucas M, Schachterle W, Oberle K, Aichele P, Diefenbach A. Dendritic cells prime natural killer cells by trans-presenting interleukin 15. *Immunity.* 2007; 26:503–517. [PubMed: 17398124]
 45. Tasca S, Tambussi G, Nozza S, Capiluppi B, Zocchi MR, Soldini L, Veglia F, Poli G, Lazzarin A, Fortis C. Escape of monocyte-derived dendritic cells of HIV-1 infected individuals from natural killer cell-mediated lysis. *Aids.* 2003; 17:2291–2298. [PubMed: 14571180]
 46. Mavilio D, Lombardo G, Kinter A, Fogli M, La Sala A, Ortolano S, Farschi A, Follmann D, Gregg R, Kovacs C, Marcenaro E, Pende D, Moretta A, Fauci AS. Characterization of the defective interaction between a subset of natural killer cells and dendritic cells in HIV-1 infection. *J. Exp. Med.* 2006; 203:2339–2350. [PubMed: 17000867]
 47. Jinushi M, Takehara T, Kanto T, Tatsumi T, Groh V, Spies T, Miyagi T, Suzuki T, Sasaki Y, Hayashi N. Critical role of MHC class I-related chain A and B expression on IFN- α -stimulated dendritic cells in NK cell activation: impairment in chronic hepatitis C virus infection. *J. Immunol.* 2003; 170:1249–1256. [PubMed: 12538683]
 48. Jinushi M, Takehara T, Tatsumi T, Kanto T, Groh V, Spies T, Suzuki T, Miyagi T, Hayashi N. Autocrine/paracrine IL-15 that is required for type I IFN-mediated dendritic cell expression of MHC class I-related chain A and B is impaired in hepatitis C virus infection. *J. Immunol.* 2003; 171:5423–5429. [PubMed: 14607946]
 49. Anthony DD, Post AB, Valdez H, Peterson DL, Murphy M, Heeger PS. ELISPOT analysis of hepatitis C virus protein-specific IFN- γ -producing peripheral blood lymphocytes in infected humans with and without cirrhosis. *Clin. Immunol.* 2001; 99:232–240. [PubMed: 11318595]
 50. Moretta L, Ferlazzo G, Bottino C, Vitale M, Pende D, Mingari MC, Moretta A. Effector and regulatory events during natural killer-dendritic cell interactions. *Immunol. Rev.* 2006; 214:219–228. [PubMed: 17100887]
 51. Meier UC, Owen RE, Taylor E, Worth A, Naoumov N, Willberg C, Tang K, Newton P, Pellegrino P, Williams I, Klenerman P, Borrow P. Shared alterations in NK cell frequency, phenotype, and

- function in chronic human immunodeficiency virus and hepatitis C virus infections. *J. Virol.* 2005; 79:12365–12374. [PubMed: 16160163]
52. Fauci AS, Mavilio D, Kottlil S. NK cells in HIV infection: paradigm for protection or targets for ambush. *Nat. Rev. Immunol.* 2005; 5:835–843. [PubMed: 16239902]
53. Jinushi M, Takehara T, Tatsumi T, Kanto T, Miyagi T, Suzuki T, Kanazawa Y, Hiramatsu N, Hayashi N. Negative regulation of NK cell activities by inhibitory receptor CD94/NKG2A leads to altered NK cell-induced modulation of dendritic cell functions in chronic hepatitis C virus infection. *J. Immunol.* 2004; 173:6072–6081. [PubMed: 15528343]
54. Chehimi J, Starr SE, Frank I, Andrea AD, Ma X, MacGregor RR, Sennelier J, Trinchieri G. Impaired interleukin 12 production in human immunodeficiency virus-infected patients. *J. Exp. Med.* 1994; 179:1361–1366. [PubMed: 7908324]
55. Chougnat C, Wynn TA, Clerici M, Landay AL, Kessler HA, Rusnak J, Melcher GP, Sher A, Shearer GM. Molecular analysis of decreased interleukin-12 production in persons infected with human immunodeficiency virus. *J. Infect. Dis.* 1996; 174:46–53. [PubMed: 8656012]
56. Horlock C, Shakib F, Mahdavi J, Jones NS, Sewell HF, Ghaemmaghami AM. Analysis of proteomic profiles and functional properties of human peripheral blood myeloid dendritic cells, monocyte-derived dendritic cells and the dendritic cell-like KG-1 cells reveals distinct characteristics. *Genome Biol.* 2007; 8:R30. [PubMed: 17331236]

Figure 1.

Poly (I:C) induced CD3 depleted PBMC IFN- γ is impaired in HIV infection, and this activity is associated with IL-12. Panel A: PBMC were cultured in the absence (media) or presence of poly (I:C) (50 μ g/ml) or IL-12 (1 ng/ml) for 20 h. Cells were stained for cell surface expression of CD3 and CD56, then permeabilized and stained for intracellular expression of IFN- γ . NK cell IFN- γ expression was analyzed by flow cytometric analysis, gating on CD3⁺CD56⁺ cells. Proportions of CD3⁺CD56⁺ cells expressing IFN- γ are shown in the upper right quadrant of each plot. Data shown for this subject's sample are representative of 3 separate experiments. Panels B and C: CD3 depleted PBMC from untreated HCV infected ($n=15$), HIV infected ($n=9$), HCV/HIV infected ($n=15$), and Control ($n=15$) subjects were stimulated with poly (I:C) (Panel B) or IL-12 (Panel C) for 20 h at 37 °C, and analyzed for IFN- γ secretion by ELISPOT assay. Y axis represents the numbers of IFN- γ spot forming units/300,000 cells above background (media stimulated cells). Media stimulated cell background ranged from 0–5 SFU/300,000 cells. Panel D: IL-12 production by PBMC in response to Poly (I:C) is associated with Poly (I:C) induced CD3 depleted PBMC IFN- γ .

Figure 2.

Poly (I:C) induced IFN- γ production is dependent on MDC, and in part dependent on IL-12. Panel A: CD3 depleted PBMC from healthy control subjects ($n=5$) were stimulated with Poly(I:C) (50 $\mu\text{g}/\text{ml}$, 20 h 37 $^{\circ}\text{C}$) in the presence or absence of IL-12 blocking antibody and IFN- γ production was measured by ELISPOT assay. Proportion of activity that remains after blocking is represented for each subject (S1–S5). As a control, IL-12 induced IFN- γ was completely blocked by IL12 blocking antibody and not by isotype control antibody (*not shown*). Panel B: Poly (I:C) induced CD3-PBMC IFN- γ as measured by ELISPOT assay is dependent on MDC. CD3 depleted PBMC from control subjects ($n=3$) were stimulated with

Poly(I:C) (50 µg/ml for 20 h at 37 °C) in the presence (intact system) or absence of MDC (greater than 75% MDC depletion) and IFN- γ production was measured by ELISPOT assay. Panel C: PBMC, NK cells, monocytes, and DC (combined DC) were prepared from control subjects ($n=3$) and were analyzed for poly (I:C) induced IL-12 production per 50,000 cells by ELISA. Mean and standard deviation shown.

Figure 3.

MDC yield from HIV infected subjects is reduced. PBMC were prepared from 100 ml blood samples obtained from HCV infected, HIV infected and healthy control subjects. MDC were isolated from PBMC by bead isolation method. Yield of MDC per 1×10^8 PBMC is shown for HCV, HIV and control subjects.

Figure 4.

On a per cell basis poly (I:C) dependent, MDC dependent NK IFN- γ is intact in HCV and HIV infection. Panel A: Healthy control subject NK cells (100,000/well) were cultured in the presence of media alone, IL-12 (1 ng/ml) or Poly(I:C) (50 μ g/ml) 20 h 37 °C and IFN- γ producing cell frequency for IL-12 and poly (I:C) above that observed in media alone cultures was measured by ELISPOT method. Panel B: A representative example of an assay performed for a given subject in the presence or absence of MDC, in the presence or absence of NK target cells (K562 cells) at 5:1 E:T ratio is shown. Panel C: NK cells (100,000/well)

were co-cultured in the presence or absence of autologous MDC (20,000/well) and analyzed for poly (I:C) (50 µg/ml, 20 h 37 °C) induced IFN- γ by ELISPOT method. NK IFN- γ dependent on the presence of MDC in response to poly (I:C) was determined by subtracting poly (I:C) induced NK IFN- γ in the absence of MDC from that in the presence of MDC. Panel D: NK (100,000 cells/well) IFN- γ production dependent on MDC in response to poly (I:C), in the presence of NK target cells (20,000 K562/well). Panel E: poly (I:C) induced MDC dependent NK Granzyme B production in the presence of NK target cells (K562).

Table 1

Clinical characteristics for subject samples analyzed in unfractionated assays

	HCV	HIV	HCV-HIV
Number	15	15	9
Plasma HCV RNA level (IU/ml)	2,200,000 (1,160,000, 3,770,000)	ND	1,204,946 (615,478, 2,588,443)
Plasma HIV RNA level (C/ml) ^a	ND	50 (50, 34500)	50 (50, 225)
ALT (u/l)	54 (35, 117)	38 (29, 48)	52 (44, 71)
CD4 cell count (×10 ⁶ /L)	ND	578 (285, 851)	379 (302, 498)
HCV genotype 1	10	ND	4
HCV genotype 2/3	5	ND	0
HCV genotype other	0	ND	1
HCV genotype unknown	0	ND	4

Values for HCV, HIV, ALT, and CD4 represent median (interquartile range).

^aLower limit of assay for HIV detection is 50 C/ml.