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2B4$^+$ CD8$^+$ T cells play an inhibitory role against constrained HIV epitopes

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ABSTRACT

Cytotoxic T cells play a critical role in the control of HIV and the progression of infected individuals to AIDS. 2B4 (CD244) is a member of the SLAM family of receptors that regulate lymphocyte development and function. The expression of 2B4 on CD8$^+$ T cells was shown to increase during AIDS disease progression. However, the functional role of 2B4$^+$ CD8$^+$ T cells against HIV infection is not known. Here, we have examined the functional role of 2B4$^+$ CD8$^+$ T cells during and after stimulation with HLA B14 or B27 restricted HIV epitopes. Interestingly, IFN-γ secretion and cytotoxic activity of 2B4$^+$ CD8$^+$ T cells stimulated with HIV peptides were significantly decreased when compared to influenza peptide stimulated 2B4$^+$ CD8$^+$ T cells. The expression of the signaling adaptor molecule SAP was downregulated in 2B4$^+$ CD8$^+$ T cells upon HIV peptide stimulation. These results suggest that 2B4$^+$ CD8$^+$ T cells play an inhibitory role against constrained HIV epitopes underlying the inability to control the virus during disease progression.

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1. Introduction

An effective cytotoxic T lymphocyte (CTL) response is essential for the immune system to control HIV [1,2]. A unique set of seropositive individuals that are capable of containing HIV infection and delaying the progression to AIDS, termed as Long Term Non-Progressors (LTNPs) [3], have been shown to have potent anti-HIV CD8$^+$ T cell responses [1,2]. Many LTNPs possess human leukocyte antigen (HLA) B14 or B27 and several studies have shown that individuals that express B14 or B27 have an overall low severity of disease during viral infection. This is likely due to its ability to present epitopes of the virus that are shared among different strains and present it with complex stability [4]. Furthermore, this presentation causes heightened activation of killer T cells that can kill more viral particles. Thus, allowing for immune control of HIV in infected individuals to control viral replication for long periods of time, although not completely eliminating the virus.

HIV possesses many genes that allow it to infect, evade and replicate in a host. The group-specific antigen (Gag) gene in HIV codes for many structural proteins needed for the virion itself. Although, Gag is a late structural protein in the replication cycle of HIV, it has been shown that Gag-specific CD8$^+$ T cells recognize infected cells around 2 h post-infection with SIV-mac239 and are able to eliminate infected cells as early as 6 h after infection [5]. Conversely, Tat and Env proteins were not able to generate this heightened early response. Specifically we are interested in the p17 and p24 Gag region which makes up the protective matrix and viral capsid, respectively. It has been suggested that the resistance caused by HLA B14 and B27 are due to strong CTL responses against the p17 and p24 Gag proteins [1,6]. However, in non-progressors that are not linked with HLA B27, a p24 specific proliferative response was associated with control of HIV when combined with CTL, additionally highlighting the importance of p24 targeting [7].

2B4 (CD244) is a member of the signaling lymphocyte activation molecule (SLAM/CD150) family of receptors that regulate immune responses [8,9]. 2B4 was first identified in mice as an NK cell activating receptor [10]. However, it is now well established that 2B4 can function as an activating or inhibitory receptor in both mice and human [11,12]. In addition to NK cells, 2B4 is expressed on basophils, eosinophils, monocytes and a subpopulation of CD8$^+$ T cells [13,14]. In human NK cells, this receptor usually acts as an activator of cytotoxic function and IFN-γ secretion, although it has been found to act as an inhibitory receptor when expressed at high levels [8,11]. CD48 is the physiological ligand for 2B4 and its interaction causes cell proliferation when induced between NK cells or with nearby T cells [11,15,16]. 2B4$^+$ CD8$^+$ T cells represent a population of activated or memory T cells, usually about 50% or less of the total amount of the whole CD8$^+$ T cell population [17,18]. Little is known about this receptor's functional role in

Abbreviations: BLCL, B lymphoblastoid cell lines; Gag, group-specific antigen; mDC, monocytes-derived Dendritic cells; MCM, monocyte conditioned media; SAP, SLAM–associated protein; APC, antigen presenting cell.

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HIV infection and the progression to AIDS. Interestingly, the levels of $2B4^+$ $CD8^+$ T cells have been shown to increase during the course of HIV disease [19]. The expression of the 2B4 ligand CD48 is downregulated in HIV infected cells [20]. During HIV infection, NK cells have been shown to upregulate 2B4 but have lowered killing activity of HIV infected cells, despite the infected cell downregulation of MHC molecules [21]. SLAM–associated protein (SAP), an intracellular adaptor molecule that mediates signal transduction through 2B4, is expressed in NK and T cells. Previous studies highlight the importance of this signaling molecule in the activating function of 2B4 [17,22]. Examination of $2B4^+$ $CD8^+$ T cells in relation to Human T-Lymphocyte Virus-1 (HTLV-1) demonstrate that 2B4 and the SAP pathway may be involved in cytotoxic activity of $CD8^+$ T cells [23]. Blocking 2B4 or knocking down SAP expression resulted in inhibition of killing activity, implying that 2B4 is involved in the recognition and killing of HTLV-1 infected cells [23].

In this study, we generated an *in vitro* method using HIV negative HLA B14 and B27 donors in order to compare the responses of $2B4^+$ and $2B4^-$ T cell populations after priming with conserved HIV antigens with known affinity for the corresponding HLA types. We observed upregulation of the 2B4 receptor in both populations of cells. However, IFN-γ secretion and cytotoxic activity by the $2B4^+$ $CD8^+$ T cell population that was stimulated with HIV peptide was significantly decreased when compared to influenza peptide stimulated controls. Also, SAP was downregulated in $2B4^+$ $CD8^+$ T cells after HIV peptide pulsed antigen presenting cell (APC) stimulation. This suggests that 2B4 may have a key role in the suppressed immune function of $2B4^+$ $CD8^+$ T cells during HIV infection.

2. Materials and methods

2.1. Identification of HLA B27 supertype donors and cell lines

Initial screening of subjects was done by obtaining buccal swabs from healthy volunteers. These samples were HLA typed at Tepnel Lifecodes, Stamford, CT. Four volunteers were identified as having HLA B14 or B27. Blood samples were obtained from these donors and the respective B lymphoblastoid cell line (BLCL) were generated at the tissue culture facility at UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC. Arbitrarily, BLCL-2 expresses class I HLA B14 and B8, BLCL-3 expresses class I HLA B27 and B44, and BLCL-4 expresses class I HLA B27 and B8. All cell lines were cultured in RPMI 1640 supplemented with 10% FBS and antibiotic (Invitrogen, Carlsbad, CA).

2.2. Conserved HIV peptides

Synthetic HIV peptides were selected using the Los Alamos database. These peptides were chosen based on known affinity for HLA B14 and B27. All peptides are from conserved regions of the HIV protective matrix and capsid structural protein, p17 and p24 Gag, respectively. Synthetic peptides were synthesized by Celtek Peptides, Nashville, TN. Six conserved HIV peptides including escape variants and one influenza virus peptide used in this study are listed in Table 1.

2.3. Monocyte induced differentiation to dendritic cells

Whole blood was obtained from the same donors previously used to prepare the BLCL. PBMC's were purified by ficol separation using a histopaque gradient and monocytes were isolated by magnetic activated cell sorting (MACS) using a monocyte isolation kit (Miltenyi Biotec, Auburn, CA) or by allowing adherence to a polyethylene plate as previously described [24]. Monocytes were differentiated into dendritic cells by culturing for 10 days in the presence of 800 U/ml recombinant human GM-CSF and 500 U/ml recombinant human IL-4 (R&D systems, Minneapolis, MN). Monocyte conditioned media (MCM) was added for dendritic cell maturation on day 7 at a 50% concentration. MCM was made as previously described [24].

Table 1
Influenza and HIV peptides and escape variants.

Name	Epitope sequence	Binding	Region of virus
FL-8	F L K E KG G L	Influenza peptide	
DE-10-1	D R F Y K T L R A E	B14	p24 gag 166–174
DE-10-2	D R F Y K ***I*** L R A E	Escape variant	
IK-9	I R L R P G G K K	B27	p17 gag 19–27
IY-11	I L G L N K I V R M Y	B7, presumed to bind B27	p24 gag 135–145
KK10-1	K R W I I L G L N K	B27	p24 gag 131–140
KK10-2	K R W I I ***M*** G L N K	Escape variant	

Bold italicized amino acids denote mutations.

2.4. Dendritic cell presentation and $CD8^+$ T cell activation

$CD8^+$ T cells were isolated from whole blood by MACS using a $CD8^+$ T cell kit (Miltenyi Biotec, Auburn, CA). After incubation with anti-2B4 mAb (C1.7, Beckman Coulter, Brea, CA), $2B4^-$ and $2B4^+$ T cell populations were separated by Cytopeia influx cell sorting. After 10 days incubation, monocyte-derived dendritic cells (mDC), discussed above, were cultured without (deemed "no peptide" cultured) or with 50 μg/ml of each peptide (Table 1) and 3 μg/ml β_2-Microglobulin (β_2M) (Calbiochem, Gibbstown, NJ) in AIM-V medium (Invitrogen, Carlsbad, CA) for 2–4 h at 37 °C. After peptide pulsing, mDC's were treated with 100 μg/ml mitomycin C (Sigma, St. Louis, MO), immediately washed and resuspended into 4+RPMI with 10% human AB serum. $CD8^+$ T cells were resuspended and layered onto the mDC's with a final volume of 1.5 ml. 10 ng/ml IL-7 was added to the cultures on day 1 and 10 units/ml IL-2 was added on day 2 (R&D systems, Minneapolis, MN) and cultured for 8 days at 37 °C. After the first 8 days stimulation, new mDC's were added in a similar fashion for the second stimulation and cultured for another 8 days. After each stimulation, supernatant was harvested from each well and an Interferon-γ Enzyme Linked ImmunoSorbent Assay (IFN-γ ELISA) was performed (BD Biosciences, San Jose, CA).

2.5. Cell surface expression of 2B4

$2B4^-$ and $2B4^+$ $CD8^+$ T cells were harvested from each stimulated population, as discussed previously, after two rounds of stimulation. After incubation with anti-2B4 mAb (C1.7), flow cytometry was performed and 2B4 expression was determined by mean fluorescence intensity (MFI).

2.6. Cytotoxicity assay comparison of $2B4^-$ and $2B4^+$ $CD8^+$ T cells

After two stimulations with APC's, a 4 h cytotoxicity assay was performed. $CD8^+$ T cells were harvested and used to make serial dilutions such that there would be an effector to target ratio of 20:1. For making target cells, BLCLs discussed previously were used and were pulsed at a concentration of 50 μg/ml with each of the individual peptides (same as used for $CD8^+$ T cell priming) overnight in AIM-V medium at 37 °C prior to performing this assay. After pulsing overnight, BLCLs were resuspended into 4+RPMI with 10% human AB serum and incubated for 90 min at 37 °C with radioactive sodium chromate (^{51}Cr) (Perkin Elmer, Waltham, MA) at a concentration of 1/5 chromate to 4/5 medium and were