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IL-4 and IL-13 mediated down-regulation of CD8 expression levels can dampen anti-viral CD8$^+$ T cell avidity following HIV-1 recombinant pox viral vaccination

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ABSTRACT

We have shown that mucosal HIV-1 recombinant pox viral vaccination can induce high, avidity HIV-specific CD8$^+$ T cells with reduced interleukin (IL)-4 and IL-13 expression compared to, systemic vaccine delivery. In the current study how these cytokines act to regulate anti-viral CD8$^+$ T, cell avidity following HIV-1 recombinant pox viral prime-boost vaccination was investigated. Out of a panel of T cell avidity markers tested, only CD8 expression levels were found to be enhanced on, KdGag$_{197–205}$ (HIV)-specific CD8$^+$ T cells obtained from IL-13$^{-/-}$, IL-4$^{-/-}$ and signal transducer and, activator of transcription of 6 (STAT6)$^{-/-}$ mice compared to wild-type (WT) controls following, vaccination. Elevated CD8 expression levels in this instance also correlated with polyfunctionality, (interferon (IFN)-γ, tumour necorsis factor (TNF)-α and IL-2 production) and the avidity of HIVspecific CD8$^+$ T cells. Furthermore, mucosal vaccination and vaccination with the novel adjuvanted IL-13 inhibitor (i.e. IL-13Rα2) vaccines significantly enhanced CD8 expression levels on HIV-specific CD8$^+$, T cells, which correlated with avidity. Using anti-CD8 antibodies that blocked CD8 availability on CD8$^+$, T cells, it was established that CD8 played an important role in increasing HIV-specific CD8$^+$ T cell avidity and polyfunctionality in IL-4$^{-/-}$, IL-13$^{-/-}$ and STAT6$^{-/-}$ mice compared to WT controls, following vaccination. Collectively, our data demonstrate that IL-4 and IL-13 dampen CD8 expression levels on anti-viral CD8$^+$ T cells, which can down-regulate anti-viral CD8$^+$ T cell avidity and, polyfunctionality following HIV-1 recombinant pox viral vaccination. These findings can be exploited to, design more efficacious vaccines not only against HIV-1, but many chronic infections where high, avidity CD8$^+$ T cells help protection.

1. Introduction

Even though effective anti-retroviral drugs are currently available against HIV-1 [1], developing a vaccine still remains a major priority for slowing down the progression of HIV-1 incidences worldwide. Unfortunately, this has been a daunting task given the capacity of HIV-1 to maintain latency and mutate rapidly allowing it to evade the immune system [2]. Furthermore, many of the past HIV-1 vaccines that have elicited protective outcomes in animals have failed to do so in humans [3–8].

The induction of high avidity anti-viral CD8$^+$ T cells responses is thought to be critical for effective protective immunity against viruses including HIV-1 [9,10]. Various molecules that facilitate cell adhesion (e.g. CD2 and CD11a), cognate peptide-major histocompatibility class I complexes (pMHC-I) recognition (i.e. densities of CD8 and T cell receptor (TCR) interaction) and intracellular signalling such as lck have been known to regulate T cell avidity [11–14]. High avidity anti-viral CD8$^+$ T cells can recognize and

Abbreviations: IL, interleukin; TCR, T cell receptor; IFN, interferon; TNF, tumor necrosis factor; WT, wild-type; STAT6, signal transducer and activator of transcription 6; pMHC-I, peptide-major histocompatibility class I complexes; i.n., intranasal; i.m., intramuscular; IL-13Rα2, IL-13 receptor α 2; FPV-HIV, fowlpox virus encoding HIV-1 antigens; VV-HIV, vaccinia virus encoding HIV-1 antigens; IL-13 inhibitor vaccines, FPV-HIV or VV-HIV encoding murine soluble IL-13Rα2; VV-WR, vaccinia virus Western Reserve strain; i.p., intraperitoneal; RBC, red blood cell; BD, Beckton Dickinson; PBS, phosphate buffer saline; FCS, fetal calf serum; ICS, intracellular cytokine stain; MFI, mean fluorescent intensity; SEM, standard error of the mean; ANOVA, analysis of variance; IFN-I, type I interferons; LCMV, lymphocytic choriomeningitis virus.

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respond to lower densities of cognate pMHC-I on virus-infected cells compared to low avidity anti-viral CD8$^+$ T cells [15]. This allows high avidity HIV-specific CD8$^+$ T cells to minimize or prevent the spread of this virus from early exposed genito-rectal mucosae to the gut where the greatest CD4$^+$ T cell depletion occur during HIV-1 infection [2,10]. High avidity anti-viral CD8$^+$ T cells are also polyfunctional (i.e. produce multiple anti-viral/survival cytokines such as IFN-γ, TNF-α and IL-2) [16,17]. Numerous studies have demonstrated that elite controllers of HIV-1 infection maintain more high avidity and polyfunctional CD8$^+$ T cells than non-controllers [18–20]. Therefore, development of vaccines that can induce high avidity HIV-specific CD8$^+$ T cells could be extremely beneficial for optimizing protective outcomes against HIV-1.

Despite the knowledge that high avidity anti-viral CD8$^+$ T cells are important for efficient virus control, the mechanisms responsible for enhancing the avidity of anti-viral CD8$^+$ T cells following vaccination is not well understood. Previous studies have shown that IL-4 and IL-13 can modulate anti-viral efficacy of CD8$^+$ T cells and control of virus infection [21–24]. We have shown that HIV-specific CD8$^+$ T cells that develop following mucosal (intranasal (i.n.)/i.n. or i.n./intramuscular (i.m.)) compared to purely systemic (i.m./i.m.) HIV-1 recombinant pox viral prime-boost vaccination have reduced expression of IL-4/IL-13, but are higher in avidity [25]. In subsequent studies, we have shown following pox viral infection and HIV-1 recombinant pox viral prime-boost vaccination that IL-4, IL-13 and STAT6 can dampen the avidity and polyfunctionality of anti-viral CD8$^+$ T cells [26,27]. Based on this knowledge we have now developed a novel HIV-1 recombinant pox viral IL-13 receptor α 2 (IL-13Rα2) adjuvanted vaccine that can be used to transiently inhibit IL-13 activity at the vaccination site [24]. This vaccine was shown to enhance the avidity/polyfunctionality of HIV-specific CD8$^+$ T cells as well as the protective efficacy [24]. Given that pox virus vectors are commonly used in vaccination studies, here we have addressed how IL-4 and IL-13 can dampen the avidity of anti-viral CD8$^+$ T cells following HIV-1 recombinant pox viral vaccination with the hope of developing more effective HIV-1 vaccines that translate into future phase I clinical trials.

2. Materials and methods

2.1. Mice

Pathogen-free 6-8 weeks old female WT, IL-4$^{-/-}$, IL-13$^{-/-}$, and STAT6$^{-/-}$ BALB/c mice were all purchased from the Australian Phenomics Facility, the Australian National University. All the mice were handled and maintained under the Australian National University Animal Experimentation and Ethics Committee approved guidelines and protocol number A2011/018.

2.2. Recombinant HIV-1 prime-boost vaccination and vaccinia virus infection

The recombinant fowlpox virus vectors encoding HIV-1 AE clade Gag, Pol and Env (FPV-HIV) and recombinant vaccinia virus encoding HIV-1 AE clade Gag and Pol (VV-HIV) used in the current study were exactly as those used previously in Ranasinghe et al. [28]. Recombinant FPV-HIV and VV-HIV co-expressing the murine soluble IL-13Rα2 (IL-13 inhibitor vaccines) were constructed exactly as described in [24].

Vaccinia virus Western Reserve (VV-WR) strain and infection with this virus was exactly as described in Wijesundara et al. [26]. Briefly, BALB/c mice were infected for 7 days intraperitoneally (i.p.) prior to isolation of splenocytes for flow cytometry analysis. For all recombinant HIV-1 prime-boost vaccinations except where indicated, pathogen free 6-8 weeks old BALB/c mice were primed i.n. with 1×10^7 PFU of FPV-HIV followed by i.m. booster vaccination with 1×10^7 PFU of VV-HIV 14 days apart as described in Ranasinghe et al. [28]. T cell responses were evaluated 14 days post booster vaccination.

2.3. Flow cytometry

Monoclonal antibodies against mouse antigens CD2 (clone RM2-5), CD11a (clone 2D7), TCRβ (clone H57–597), IFN-γ (clone XMG1.2), H-2 K^d (clone SF1-1.1) and CD8β.2 (clone 53-5.8) were all purchased from BioLegend with the exception of CD8α (clone 53-6.7), lck (clone MOL 171) and TNF-α (clone MP6-XT22), which were purchased from Becton Dickinson (BD) Biosciences. These antibodies were used as purified (not conjugated) or conjugated with fluorescein isothiocyanate, phycoerythrin, peridinin chlorophyll protein, allophycocyanin or pacific blue. Peridinin chlorophyll protein conjugated monoclonal antibody against mouse IL-2 (clone JES6-5H4) was purchased from eBioscience. All cell surface and intracellular staining procedures with these antibodies were performed as described previously [26]. Stained samples were analyzed using the BD FACScalibur or BD LSR II prior to analysis using the FlowJo Tree Star software (version 8.8.7). For each sample 2,00,000–10,00,000 events were acquired.

2.4. Tetramer staining and dissociation assays

Allophycocyanin conjugated K^{d}A52$_{75–83}$ or K^{d}Gag$_{197\text{-}205}$ tetramers were synthesized by the Biomolecular Resource Facility, the John Curtin School of Medical Research. Tetramer staining was done using red blood cell (RBC)-depleted single cell suspensions from the spleens of vaccinated mice exactly as that described in Ranasinghe et al. [25]. For blocking CD8 engagement with K^{d}Gag$_{197\text{-}205}$ tetramer, cells (2×10^6 cells/25 μl phosphate buffer saline (PBS)) were incubated with varying concentrations of purified anti-mouse CD8β.2 for 30 min at 4 °C prior to tetramer staining. When tetramer dissociation was performed, tetramer stained cells were incubated for 60 min at 37 °C + 5% CO_2 in the presence of 25 μg/ml of anti-mouse H-2 K^d (2×10^6 cells/40 μl PBS) to prevent tetramer re-binding to cells. After tetramer staining and/or dissociation, cells were washed twice using PBS + 1% foetal calf serum (FCS) prior to conducting a cell surface stain.

2.5. Peptide stimulation for intracellular cytokine staining (ICS)

H-2 K^d restricted Gag$_{197\text{-}205}$ (AMQMLKETI) peptides were synthesized at the Biomolecular Resource Facility, the John Curtin School of Medical Research. K^{d}Gag$_{197\text{-}205}$ peptide stimulation of splenocytes was performed similar to that described previously [26]. Briefly, 4×10^6 RBC-depleted splenocytes resuspended in 200 μl of RPMI + 10% FCS were incubated for 1 h at 37 °C + 5% CO_2 in the presence or absence of 0.1 μg/ml of K^{d}Gag$_{197\text{-}205}$ peptide. Subsequently, each culture was incubated with 1× brefeldin A (eBioscience) for further 4 h at 37 °C + 5% CO_2 prior to cell surface and intracellular staining. When CD8 blocking was performed, cells were incubated with 1 μg/ml of purified anti-mouse CD8β.2 prior to and during K^{d}Gag$_{197\text{-}205}$ peptide stimulation.

2.6. Statistical analysis

Mean fluorescent intensity (MFI) values and flow cytometry plots were obtained following analysis of data using FlowJo Tree Star software (version 8.8.7). The data plotted in all the graphs represent the mean and the error bars depict the standard error of the mean (SEM). Statistical significance of the data and the p values were calculated using the Graph InStat software (version 3.10). In all statistical significance analysis, a Student's t-test or one-way