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Human CD8+ T cells mediate protective immunity induced by a human malaria vaccine in human immune system mice

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ABSTRACT

A number of studies have shown that CD8+ T cells mediate protective anti-malaria immunity in a mouse model. However, whether human CD8+ T cells play a role in protection against malaria remains unknown. We recently established human immune system (HIS) mice harboring functional human CD8+ T cells (HIS-CD8 mice) by transduction with HLA-A*0201 and certain human cytokines using recombinant adeno-associated virus-based gene transfer technologies. These HIS-CD8 mice mount a potent, antigen-specific HLA-A*0201-restricted human CD8+ T-cell response upon immunization with a recombinant adenovirus expressing a human malaria antigen, the *Plasmodium falciparum* circumsporozoite protein (PfCSP), termed AdPfCSP. In the present study, we challenged AdPfCSP-immunized HIS-CD8 mice with transgenic *Plasmodium berghei* sporozoites expressing full-length PfCSP and found that AdPfCSP-immunized (but not naïve) mice were protected against subsequent malaria challenge. The level of the HLA-A*0201-restricted, PfCSP-specific human CD8+ T-cell response was closely correlated with the level of malaria protection. Furthermore, depletion of human CD8+ T cells from AdPfCSP-immunized HIS-CD8 mice almost completely abolished the anti-malaria immune response. Taken together, our data show that human CD8+ T cells mediate protective anti-malaria immunity *in vivo*.

1. Introduction

Malaria is a mosquito-borne infectious disease caused by parasitic protozoans of the genus *Plasmodium*. Although medications and mosquito control efforts have limited the disease, malaria is still pandemic, with 198 million cases occurring in 2013, resulting in 584,000 fatalities [1]. These data underscore the need for new methods to control this disease, including more effective vaccines.

Most vaccine efforts are directed against the pre-erythrocytic stages [sporozoites (Spz) and liver stages], and blood stages [2]. The finding that vaccination with radiation-attenuated sporozoites (IrSpz) can induce complete protection (i.e., sterile immunity) against malaria infection not only in experimental animals but also in man [3–7] demonstrated the feasibility of effective vaccination against this disease. A number of mouse studies to date using *Plasmodium yoelii* and *Plasmodium berghei* parasites for challenge have shown that protective immunity against pre-erythrocytic stages is mediated in part by T cells, particularly CD8+ T cells. Firstly, the major role for CD8+ T cells was shown by studies in which *in vivo* depletion of CD8+ T cells abrogated Spz-induced protective immunity in mice [8,9]. Secondly, the adoptive transfer of CD8+ T-cell clones specific for the immunodominant CD8+ T-cell epitope of the *P. berghei* or *P. yoelii* circumsporozoite protein (CSP), a major Spz antigen, confers protection against Spz challenge in naïve mice [10–12]. Using transgenic mice expressing a T-cell receptor (TCR), based on the TCR sequence of CD8+ T cells recognizing a CD8+ T-cell epitope present in *P. yoelii* CSP (PyCSP), transgenic CD8+ T cells were shown to mediate protection against malaria [13]. Finally, a single immunizing dose of a recombinant adenovirus expressing the PyCSP, AdPyCSP, has been shown to induce a potent protective

Abbreviations: Ad, adenovirus; AAV9, adeno-associated virus serotype 9; CSP, circumsporozoite protein; FL, full-length; HIS, human immune system; Pf, *Plasmodium falciparum*; Py, *Plasmodium yoelii*; TCR, T-cell receptor.

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anti-malarial immunity, which was mediated primarily by CD8+ T cells [14].

Beyond mouse model, Hoffman's group has recently shown that intravenous (IV) immunization of IrSpz of *Plasmodium falciparum*, PfSPZ vaccine, is very effective in inducing a high frequency of malaria-specific CD8+ T cells in the liver of nonhuman primates [15]. More recently the same group showed that immunization of multiple doses of their IrPfSPZ vaccine by IV induced a high level of PfSPZ-specific T-cell responses, including that of CD8+ T cells, and conferred protection in six out of six (100%) human vaccinees against malaria challenge [16]. Another recent study has shown that the administration of live *P. falciparum* Spz by bites of infected mosquitoes, followed by chloroquine treatment induced significant malaria-specific pluripotent effector memory T-cell responses in vaccinated volunteers and protected all of them (10 out of 10) upon malaria challenge [17]. With regards to human malaria vaccines based on viral vectors, a small number have entered human clinical trials in recent years. In a phase I clinical trial, 15 volunteers were primed with plasmid DNA encoding *P. falciparum* CSP (PfCSP) and apical membrane antigen-1 and then boosted with human adenovirus serotype 5 (Ad5) expressing the same antigens. This DNA priming/adenovirus boost immunization regimen induced sterile protection in four (27%) vaccinated subjects [18]. In a phase IIa clinical trial, vaccination using a priming-boost regimen based on chimpanzee adenovirus and modified Ankara vaccinia virus, both expressing *P. falciparum* thrombospondin adhesive protein fused to multiple epitopes derived from several malaria antigens, induced sterile protection in 21% (3 out of 14) of subjects and delayed patency in 36% (5 out of 14) of subjects [19]. Although the degree of protection in these trials was modest, both trials exhibited a trend toward a higher-level vaccine-induced CD8+ T-cell response in protected individuals [18,19]. Finally, it has been shown that the PfCSP also contains CD8+ T cell epitopes [20,21] and can elicit a potent CD8+ T-cell response in humans upon immunization with various human malaria vaccines, including PfSPZ and AdPfCSP [22–26].

In a previous study, we established a human immune system (HIS) mouse model by transducing genes encoding human HLA-A*0201 and human cytokines using a recombinant adeno-associated virus serotype 9 (AAV9) vector [27]. These mice express functional HLA-A*0201-restricted human CD8+ T cells, and were therefore designated HIS-CD8 mice. In the present study, the role of vaccine-induced human CD8+ T cells in mediating protective immunity against malaria was investigated in HIS-CD8 mice.

2. Materials and methods

2.1. Ethics statement

All animal experiments were carried out in strict accordance with the Policy on Humane Care and Use of Laboratory Animals of the United States Public Health Service. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at The Rockefeller University. Mice were euthanized using CO_2, and every effort was made to minimize suffering. Human fetal liver samples were obtained via a non-profit partner (Advanced Bioscience Resources, Alameda, CA). As no information was obtained that would identify the subjects from whom the samples were derived, Institutional Review Board approval for their use was not required, as previously described [27].

2.2. Mice

NOD.Cg^{tm1Unc} *Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice exhibiting features of both severe combined immunodeficiency mutations and interleukin (IL)-2 receptor gamma-chain deficiency were purchased from Jackson Laboratories (Bar Harbor, ME) and maintained under specific pathogen-free conditions in the animal facilities at The Rockefeller University Comparative Bioscience Center.

2.3. Generation of HIS-CD8 mice

Recombinant AAV9 (rAAV9) vectors encoding human IL-3, IL-15, GM-CSF, and HLA-A*0201 were constructed as previously described [27]. Four-week-old NSG mice were transduced with rAAV9 encoding HLA-A*0201 by intrathoracic (IT) injection and with rAAV9 encoding HLA-A*0201 and AAV9 encoding human IL-3, IL-15, and GM-CSF, by intravenous (IV) injection, as previously described [27]. Two weeks later, mice were subjected to 150-Gy total body sub-lethal irradiation for myeloablation, and several hours later, each transduced, irradiated mouse was engrafted intravenously with 1×10^5 HLA-A*0201+ matched, CD34+ human hematopoietic stem cells (HSCs). CD34+ HSCs among lymphocytes derived from HLA-A*0201+ fetal liver samples were isolated using a Human CD34 Positive Selection kit (STEMCELL TECHNOLOGIES Inc. Vancouver, BC, Canada) [28]. At 14 weeks after HSC engraftment, the reconstitution status of human CD45+ cells in the blood of HIS-CD8 mice was determined by flow cytometric analysis, as previously described [27].

2.4. Recombinant adenovirus and transgenic parasites

A recombinant adenovirus serotype 5 (Ad5) expressing a green fluorescent protein (GFP) alone in its transgene, AdGFP, was previously constructed [29]. A recombinant Ad5 expressing *P. falciparum* CSP (AdPfCSP) was also previously constructed as described [29]. Briefly, a gene encoding a full length PfCSP was codon-optimized and synthesized, followed by being inserted into pShuttle-CMV, which was then used to make the recombinant AdPfCSP. HIS-CD8 mice were immunized with 5×10^{10} AdPfCSP virus particles [27,29]. A transgenic *P. berghei* sporozoite expressing full-length *P. falciparum* CSP (FLPfCSP/Pb sporozoite) was generated as previously described [30,31] and maintained at the Insectary Core Facility of New York University.

2.5. Intra-cellular cytokine staining (ICS)

Spleens were harvested from HIS-CD8 mice 10 days after vaccination with AdPfCS or AdGFP or from naïve HIS-CD8 mice. After isolation of lymphocytes, the cells were counted and used for ICS upon stimulation with synthetic peptides corresponding to the A2-restricted CD8+ T-cell epitopes of the PfCSP (YLNKIQNSL, KLRKPKHKKL and SLKKNSRSL) [20,21]. Briefly, Lymphocytes were stimulated for 4–6 h using a pool of the synthetic peptides listed above or none (as a negative control) in the presence of brefeldin at 37 °C with 5% CO_2. ICS assays were performed as previously described [16]. Briefly, after blocking with the anti-mouse CD16/CD32 antibody, lymphocytes were stained for surface markers with antibodies against CD45, CD3, and CD8. Next, lymphocytes were permeabilized with perm/wash solution (BD Biosciences, San Jose, CA), stained with the FITC-labeled anti-human IFN-γ antibody, fixed with 1% paraformaldehyde, and analyzed using a BD LSR II (BD Biosciences).

2.6. Staining with HLA-A*0201 tetramer loaded with YLNKIQNSL peptide

An Allophyocyanin-labeled human HLA-A*0201 tetramer loaded with the peptide YLNKIQNSL, corresponding to the PfCSP CD8+ T-cell epitope [20,21], was provided by the NIH Tetramer Core Facility. HIS-CD8 mice were immunized with AdPfCSP, and 10 days later, the mice were challenged with 2×10^4 live

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