Simian Immunodeficiency Virus Infection of CD4+ T Cell Subsets and Its Impact on B Cell Responses in Early Infection

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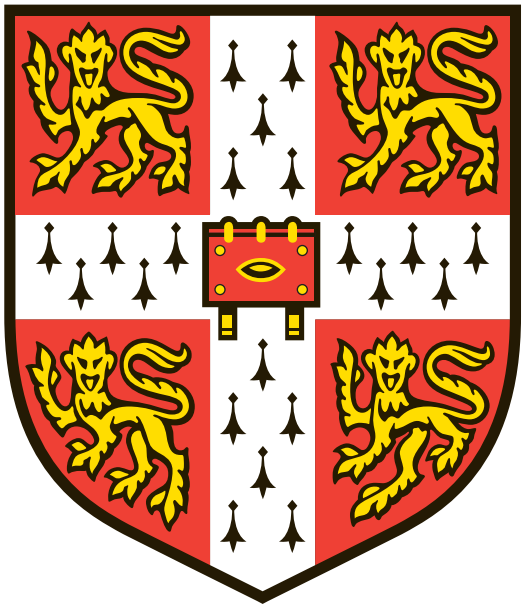

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Abstract

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Human immunodeficiency virus (HIV) disease is marked by infection and loss of CD4+ T cells and by a gradual crippling of the immune system including the absence of an effective humoral response in a majority of individuals. The model system of simian immunodeficiency virus (SIV) infection of rhesus macaques offers an opportunity to study both the immunology of early infection and immune responses in secondary lymphoid organs. In this thesis, I examine SIV evolution and infection of CD4+ T cell subsets and its impact on the SIV-specific immunoglobulin response.

Follicular helper T cells (TFH), located in secondary lymphoid organs, are thought to be a major target cell for HIV/SIV infection. We showed that during acute infection, multiple subpopulations of memory CD4+ T cells are equally infected, but productively infected cells are largely restricted to follicular T cells in chronic infection. We measured SIV infection using DNA qPCR to quantify proviral infection, and RNA qRT-PCR to quantify spliced and unspliced cell-associated RNA. Next, we used RNA probes in conjunction with flow cytometric phenotyping panels to measure the frequency of individually infected cells in memory CD4+ T cell compartments. The viral protein nef down-regulates CD4 in the early phase of cellular infection, and we show that CD3+ T cells with dim CD4 staining have the highest copy numbers of SIV RNA. Finally, we performed phenotypic analysis of CD4+ T cell subsets in uninfected, acutely infected, and chronically infected macaques using flow cytometry panels of cytokine markers.

HIV/SIV infection of TFH may impact the development of the humoral response, as early antibodies to HIV/SIV are non- or weakly neutralizing. To characterize the development of the antibody response, we performed deep sequencing of immunoglobulin heavy chains from longitudinal blood, lymph node, and bone marrow biopsies from four to 24 weeks post infection. We used a trimeric SIV gp140 protein probe to isolate SIV-specific IgG and IgM memory B cells from total memory B cells. We found that SIV protein probe specific IgG B cells increase as a percentage of lymph node and PBMC B cells over the course of infection, while SIV-specific IgM B cells are detected at lower frequencies throughout infection. SIV-specific IgG heavy chain repertoires had low scores on measures of diversity in early infection, and showed accumulation of mutations by later infection relative to total memory B cells. Common SIV-specific sequences — immunoglobulins with identical CDR3 regions and VDJ gene assignments found in different animals — represented a small but significant percentage of the total SIV-specific response.

The lifespan and location of infected cells varies greatly, and only a fraction of cells with an integrated proviral genome go on to produce replication competent virus. We examined the compartmentalization of SIV by sequencing cell-associated RNA and proviral DNA from memory CD4+ T cell subsets. We compared tissue-resident sequences to circulating plasma virus, and measure the frequency of mutations across the genomes. Finally, we examined the phylogenies within individual animals to identify the spread and evolution of viral lineages chronologically and anatomically throughout infection.

Declaration

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text.

It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University of similar institution except as declared in the Preface and specified in the text

It does not exceed the word limit of 60,000 words (excluding bibliography, figures, and appendixes) as prescribed by the Degree Committee for the Faculty of Biology at the University of Cambridge.

Dr. Kristin Boswell and Dr. Takuya Yamamoto assisted with biopsy and necropsy tissue collection. David Ambrozak performed all infectious cell sorting. All sequencing was performed either at the Wellcome Trust Sanger Institute sequencing core facility or at the Vaccine Research Center Genome Analysis Core. SIV sequencing was primers were designed with help from Dr. Shelby O'Connor at the University of Wisconsin. Dr. Swee Hoe Ong performed SIV sequence assembly.

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