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The role of $CD4^+CXCR5^+$ T follicular helper cells in the induction of antibody-producing B cells against human respiratory syncytial virus

Respiratory syncytial virus (RSV) is a ubiquitous pathogen and a major cause of lower respiratory tract infections in infants, the elderly and immunosuppressed persons. It is estimated by the WHO that RSV infects 64 million people per annum and causes 160,000 deaths, mostly in resource poor settings[1]. To date, there is no vaccine for human use, and only poorly effective antiviral drugs.

RSV is an enveloped, non-segmented negative-sense RNA virus that expresses two major surface glycoproteins, G and F. G is an attachment protein that is heavily glycosylated and highly variable, making it a doubtful choice for vaccine development. Conversely, the F protein is relatively conserved and can activate Toll-like receptor (TLR) 4 signalling[2]. It is responsible for fusion of the viral and cellular membranes and is essential for infectivity[3]. F protein is synthesized as an inactive precursor (pre-triggered) whose cleavage to a triggered conformation leads to fusion of adjoining membranes. A commercial humanised mouse monoclonal antibody (mAb) palivizumab, recognises a neutralizing epitope within the triggered F protein[4]. It is the only licenced product that has specific effects on RSV infection, but is only useful in preventing infection at high dose and, due to cost, is reserved for prophylaxis in babies at highest risk[1]. A vaccine that induces an appropriate antibody response against both pre-triggered and triggered F may therefore have the most potential for durable protective immunity against RSV.

Respiratory tract infection by RSV triggers a complex immune cascade. Initially, innate cells produce soluble mediators (e.g. interferons), which in turn promote adaptive immunity. Activated dendritic cells carry viral antigen to the regional lymph nodes and present antigenic peptides to $CD4^+$ T cells. Once primed and divided, daughter $CD4^+$ T cells migrate to the respiratory mucosa and release further mediators, recruiting eosinophils, $CD8^+$ T cells and B cells[5]. Virus-specific B cells differentiate and proliferate to form short-lived plasmablasts, which are responsible for an acute rise in antibody titre; most then rapidly apoptose, leaving only memory B cells and long-lived plasma cells to maintain humoral responses long-term. The process of affinity maturation and memory B cell formation occurs within germinal centres (GCs) that require the presence of a subset of $CD4^+$ T cells, T follicular helper (T_{FH})

cells, in order to form. In the lymphoid tissue of mice, T_{FH} cells are identified by the expression of CXCR5, PD-1, ICOS, Bcl-6 and IL-21[6]. Once primed, T_{FH} cells migrate to the B cell follicles where they form cognate interactions with B cells. The chemokine receptor CXCR5 is therefore crucial in allowing homing of T_{FH} and B cells[7]. In humans, it virtually im possible to observe or sample lymphoid structures directly, making T_{FH} cells difficult to study. However, $CXCR5^+CD4^+$ T cells are detectable in peripheral blood where they comprise three subsets (Th1, Th2 and Th17). Th2 and Th17 T_{FH} (but not Th1 T_{FH}) have been shown support antibody production *ex vivo*[7].

Since cognate interactions between $CD4^+$ T cells and B cells are essential for high affinity long-lasting antibody production, a successful vaccine should promote both T_{FH} and B cells. In this project, we will examine the epitope specificity of the human $CD4^+CXCR5^+$ T_{FH}-like cells from PBMC in parallel with the antibody-secreting cell repertoire induced following experimental human challenge with RSV. Identifying T_{FH}-like cells by FACS in human PBMCs is possible, but not easy.

However, using the expertise of Professor Openshaw (who developed the technique of intracellular cytokine analysis by FACS during a sabbatical at DNAX, California USA[8]) and the antibodies for ImmunoTools, we hope to identify these cells and to study additional expression markers. This will provide a way of identifying human T_{FH} cells in this model. It is worth highlighting that our department has made seminal contributions to the field by the use of novel FACS methods over many years [9,10,11,12,13]; we anticipate that the method will aid our understanding regarding the role of T_{FH} cells in human RSV disease, and also be of widespread use in other disease models where the study of B cell responses is important. We have a state of the art professionally run FACS facility which is part of our department.

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