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NEWS AND COMMENTARY

EBI2 regulates Tfh cells

EBI2 unlocks the door to the Tfh cell nursery

Robert Brink

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Within the ever-expanding T lymphocyte pantheon, $CD4^+$ T follicular helper (Tfh) cells occupy the unique position of being the subset that is closest to G.O.D. (generation of diversity). Initial G.O.D. among B cells occurs independent of T cells through V(D)J rearrangements in the immunoglobulin heavy and light chain variable region genes of bone marrow B-lineage precursors. However, B cells activated by foreign antigen undergo a second wave of G.O.D. when they migrate into transient lymphoid structures known as germinal centres (GCs) and undergo somatic hypermutation of rearranged variable region genes. In this case, perpetuation of GC B cells and ultimate selection of newly generated variants with increased antigen affinity depends on the delivery of cognate help from Tfh cells. Both the migration of Tfh cells into the GC and their production of stimuli specifically tailored to support GC B cells stem from a differentiation programme triggered in just a subset of antigen-activated CD4 T cells. A recent publication in *Nature* by Li *et al.*[1] provides new clues as to how the initiation of Tfh cell differentiation takes place.

These new insights centre around the chemotactic and G-protein coupled receptor EBI2 (GPR183). First identified in 1993, the function of EBI2 remained a mystery until relatively recently when it was shown to direct the migration and localisation of both B cells[2] and conventional dendritic cells (cDCs).[3] The high-affinity ligand for EBI2 is not a polypeptide, but an endogenous product of cholesterol metabolism called 7α,25-dihydroxycholesterol (7α,25-OHC).[4,5] The concentration of EBI2 ligand in specific physiological niches depends primarily on the levels in surrounding cells of the enzymes responsible for the synthesis (Cyp7b1/Ch25h) and breakdown (Hsd3b7) of 7α,25-OHC.[6] Within the spleen, the constitutive 7α, 25-OHC gradients result in the cells expressing EBI2 migrating towards the peripheral areas of the B-cell follicle.[6]

Although T cells are known to express EBI2, there is little information on the role this receptor has in directing T-cell migration following antigen activation. Similar to GC B cells, downregulation of EBI2 is important for Tfh cells to effectively migrate into GCs.[7] However, GC formation takes place 5–7 days after initial antigen contact whereas commitment to Tfh cell differentiation occurs some time beforehand. Thus, signals delivered to CD4 T cells during the first few days after antigen activation are likely to provide the key stimuli for induction of Tfh cell differentiation. Fundamental to this process is expression in antigen-activated CD4 T cells of the transcription factor Bcl6, which is triggered through engagement of the T-cell surface molecule ICOS and counteracted by the repressive transcription factor Blimp1.[8]

To determine if EBI2 has a role in Tfh differentiation, Li *et al.*[1] examined the responses of wild-type- and EBI2-deficient CD4 T cells recognising the model antigen ovalbumin (OVA). The development of activated T cells expressing the characteristic Tfh phenotype ($CXCR5^+$, $PD\text{-}1^+$) was indeed found to be reduced among EBI2-deficient T cells. Significantly, the localisation of EBI2-deficient CD4 T cells was also aberrant during the early stages of the response, with most failing to undergo normal localisation to the outer regions of the T-cell zone.[1] This mis-localisation was consistent with the inactivation of EBI2-mediated chemotaxis, as the outer T zone abuts the outer B-cell follicle where concentrations of EBI2 ligand are greatest.[6]

Although the successful completion of Tfh cell differentiation requires interaction with ICOS ligand expressed by B cells, commitment to a Tfh cell fate depends on earlier interactions with cDCs.[8] Li *et al.*[1] reasoned that the link between the early mis-localisation of activated, EBI2-deficient CD4 T cells and their impaired Tfh cell differentiation may be explained by a failure to access key signals delivered by cDCs located in the outer T-cell zone. Indeed, large numbers of cDCs were found to migrate from the splenic bridging channels to the outer T zone soon after antigen challenge, thus efficiently co-localising with wild type but not EBI2-deficient CD4 T cells. These cDCs cells not only expressed high levels of ICOS ligand, but were shown to also strongly upregulate the high-affinity IL2 receptor chain CD25.[1]

How then does the localisation of activated CD4 T cells proximal to $ICOSL^{hi}$ and $CD25^{hi}$ cDCs explain the induction of Tfh cell differentiation? Although the induction of Tfh cell differentiation by ICOS signalling is well documented, the significance of CD25 expression by the DCs was less obvious. However, IL2 signalling is known to inhibit Tfh cell differentiation, at least in part due to its induction of Blimp1 and subsequent repression of Bcl6. Li *et al.*[1] reasoned that the expression of cell surface or soluble CD25 by cDCs could act as a 'sink' for IL2 within the immediate microenvironment, thus attenuating IL2 signalling to activated CD4 T cells and promoting Tfh cell differentiation (Figure 1). Consistent with this idea, evidence for higher levels of IL2-dependent signalling (Stat5a phosphorylation, Blimp1 mRNA expression) were found among the EBI2-deficient compared with the wild-type CD4 T cells early in their responses to OVA.

Li *et al.*[1] present an interesting model for the promotion of Tfh cell differentiation

R Brink is at Immunology Division, Garvan Institute of Medical Research, Darlinghurst, New South Wales, Australia
E-mail: r.brink@garvan.org.au

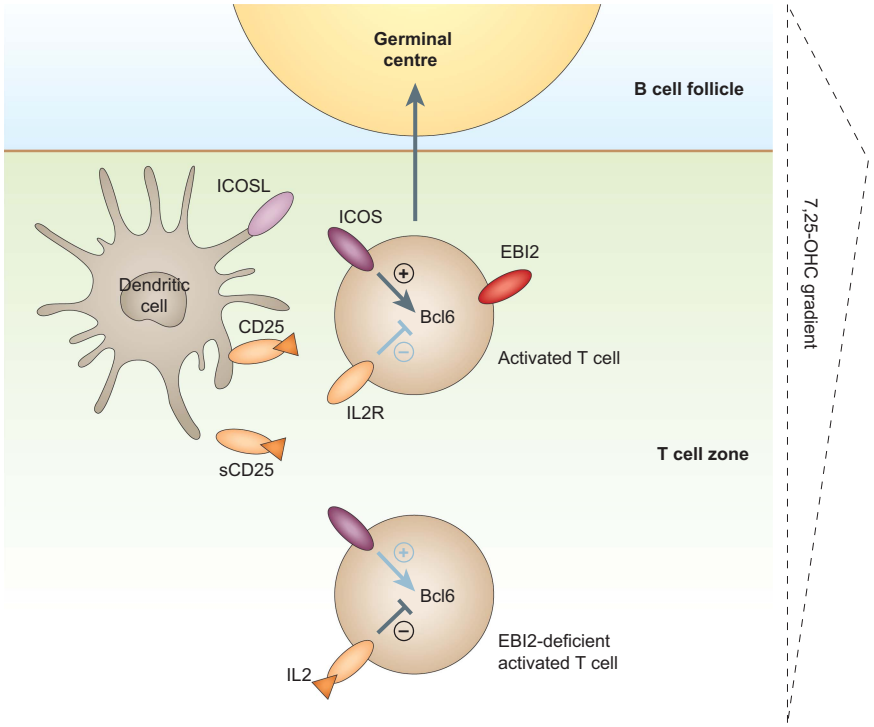

Figure 1 Model for the potentiation of Tfh cell differentiation by EBI2-mediated co-localisation with outer T-cell zone ICOSL$^+$ and CD25$^+$ cDCs. Upregulation of the chemotactic receptor EBI2 on activated CD4 T cells attracts them to the outer regions of the T-cell zone where the concentration of the EBI2 ligand is highest (7α,25-OHC). Here cDCs expressing high levels of ICOS ligand (ICOSL) and CD25 promote Bcl6 upregulation by simultaneously delivering positive signals (through ICOS) and quenching delivery of inhibitory signals (through CD25). Bcl6 expression triggers Tfh cell differentiation and migration into the germinal centre. CD4 T cells lacking EBI2 fail to localise efficiently to the outer T-cell zone and so receive strong IL2 signals that inhibit Tfh cell differentiation.

based on tightly regulated positioning of activated CD4 T cells within secondary lymphoid organs leading to the simultaneous delivery of a positive stimulus (ICOSL) and 'quenching' of a negative stimulus (IL2); (Figure 1). The concept that co-localised cells may remove extracellular stimuli has been suggested as a mechanism of action for CD25$^+$ T regulatory cells.[9] As Li *et al.*[1] point out, the significance of soluble CD25 in serum is not known and could potentially reflect a broad employment of IL2-quenching by CD25 to modulate the various activities of this extremely pleiotropic cytokine.

The importance of EBI2 in regulating immune responses is an interesting point to consider in light of the results of Li *et al.*[1] Although they reported that EBI2-deficient T cells supported reduced Tfh and GC responses, this is not always the case[7] and intact EBI2-deficient mice produce effective GC responses.[2] Although EBI2 expression is clearly not an absolute requirement for CD4 T cells to undergo Tfh cell differentiation, it seems likely that EBI2 helps to promote the efficiency of this process and therefore the effectiveness of humoral immunity in the pressing responses required to 'real world' infectious pathogens. Analysis of the role of EBI2 in such cases may further illuminate the extent to which this receptor is required for effective coordination of immune responses.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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