CD4+ Cell Development, Function & Dysregulation in Allergy and Autoimmune Diseases

The helper T (T_H) cells in the body are central to all branches of the immune system. They are very aggressive cells, due to their role as 'molecular guardians of the body' and so they must be regulated and controlled. If this regulation or self-tolerance breaks down, then this provides a window for the development of allergy, being a *"disease following a response by the immune system to an otherwise innocuous antigen"* [12], or autoimmunity, where the body's immune system targets self antigens. Before an understanding of the role of different $CD4^+$ cells in immunopathology can be achieved, an appreciation of the different characteristics and interactions of these cells must first be considered.

After preparation of antigen onto a major histocompatibility complex-II (MHC-II), an antigen presenting cell (APC), typically a dendritic cell (DC) or macrophage, will present this to a naive T_H cell. Depending on what type of pathogen has been encountered, the APC will stimulate the polarisation of the naive cell to one subset of T_H cells. This is achieved by the release of specific cytokines from the APC to the T_H cell, and the subsets of $CD4^+$ cells are each defined by their unique surface receptors, the cytokines they release and by the types of invaders they target.

The first subset to be discovered was T_H1. These target intracellular pathogens, such as *Mycobacterium spp.* and mainly utilise phagocytic and cytotoxic cells. Alongside antigen presentation, interferon-γ (IFN-γ) and interleukin-12 (IL-12) are required from the APC. IL-12Rβ2, on the $CD4^+$ cell, then induces phosphorylation of the signal transducer and activator of transcription 4 protein (STAT4) and T-bet activation. The cytokines produced by this cell type, due to STAT4 and T-bet are mostly pro-inflammatory, such as IFN-γ (which up-regulates T-bet thus forming a positive feedback loop), tumour necrosis factor β (TNFβ), IL-2 and lymphotoxin-α (LTα)[1-3].

Around this time, T_H2 cells were also discovered in response to extracellular pathogens and parasites e.g. helminths, and these cells induce humoral immunity via immunoglobulin G4 (IgG4) and IgE production. IL-4 and IL-2 induce this subset, by IL-4R directed phosphorylation of STAT6 which, with GATA-sequence binding protein 3 (GATA3), causes transcription of *IL-4*. This is the positive feedback source as IL-4 up-regulates GATA3. The cytokine profile for this cell includes IL-4 and IL-13 (which induce IgE class switching), IL-5 and Amphiregulin (for eosinophil recruitment) and IL-25 (a self-stimulating cytokine)[1-5]. Note that polymorphisms in GATA3 and IL-4Rα (at position 576. glu→arg) have been associated with hypersecretion of IgE from B cells [2].

For reasons that shall be explained later, T_H1 cells were associated with inflammatory autoimmunity, and T_H2 cells with allergies and asthma. Very recently, a new subset called T_H17 was discovered, which initiated rethinking of the T_H1-T_H2 mediation hypothesis. This type of cell is polarised in extracellular bacterial and fungal infections [2,3,6]. Like T_H1, this type of cell produces very potent pro-

inflammatory cytokines. T_H17 lineage is first induced by low levels of transforming growth factor β (TGFβ), and IL-1β/IL-6. This up-regulates IL-23R (which is not present on naive $CD4^+$ cells), sensitising the cells to IL-23, and causes cytokine production by RAR-related orphan receptor γt (RORγt) expression. These include IL-17, IL-17F, IL-21, IL-22 and TNFα. At this point, IL-21 provides the autocrine positive feedback system, causing proliferation. IL-21's importance is highlighted by the condition XSCID, where individuals have no functioning IL-21R. As B cells have high levels of IL-21R, this results in no humoral responses to infection [7]. To maintain the population of T_H17, IL-23 is required, otherwise the cells dissipate [1,2,4,6,8].

The final type of $CD4^+$ cell to be considered is the $CD4^+CD25^+$ T_{reg} cell, and this is due to the close relationship between T_H17 and induced T_{reg} (iT_{reg}) cells. The main concern of these cells is that of self tolerance, and immunity regulation. They are induced by the presence of IL-2, high levels of TGFβ but absolutely no proinflammatory cytokine presence. Instead of TGFβ , all-trans retinoic acid (ATRA) or IL-10 can be used to induce this lineage [9]. It can already be seen that there is a connection with T_H17 cells by TGFβ, and because of this RORγt is also expressed in iT_{reg}s. However, via STAT5, the master regulatory gene, *Forkhead box p3 (Foxp3),* is also transcribed, and the Foxp3 protein antagonistically binds to the RORγt protein. These cells produce suppressive cytokines such as IL-10 and IL-35, and sometimes, by IL-10, iT_{reg}s can induce plasticity of different $CD4^+$ subsets or production of IL-10 by T_H cells, thus making them self-regulatory[2,6,9].

T_{reg} cells are responsible for resisting hyperactivity of all T_H cells, including T_H1, T_H2 and T_H17. They employ the cytokines they produce and other techniques to do this, but the body has to be able to fight pathogens if actually present. Toll-like receptor (TLR) signalling is a good example of this, as such a signal suppresses T_{reg} populations to allow T_H activity. However, inability to restore homeostasis of the T_{reg}/T_H17 balance allows uncontrolled inflammation, potentially in autoimmunity, and/or lack of control of the other subsets, leading to their 'signature' immunopathologies[9].

Although all of these cells exist, and can individually proliferate as has been outlined, there are inhibitory mechanisms to this, and mostly they are intercellular from within the $CD4^+$ population. T_H1 and T_H2 are antagonists, but they both prevent T_H17 polarisation. Both IFN-γ and IL-4 prevent production of IL-23 which is absolutely necessary for T_H17 stabilisation. They inhibit each other as T-bet suppresses GATA3, whereas GATA3 downregulates STAT4 and up-regulates STAT5. STAT5, in turn, inhibits T-bet, so each has a polarisation factor that prevents polarisation factors from other lineages being expressed. Also, lack of thymic stromal lymphopoietin (TSLP) allows T_H1 dominance, whereas lack of interferon regulatory factor 2 (IRF2) or *T-bet*$^{-/-}$ had the opposite effect, directing towards T_H2. Therefore, as T_H1 and T_H2 logically cannot be co-localised, IFN-γ and IL-4 control the pivot between cell mediation and humoral mediation[1,2,4,6,8].

In addition to this, T_H17 is regulated by iT_{reg} cells, but iT_{reg} cells are regulated by T_H17 also, due to the fact that they are induced by common factors, representing a physiological balance. To prevent the cell being directed to a T_H17 cell, IL-2, via STAT5, causes *Foxp3* expression, and the section of the protein encoded by exon 2 of the *Foxp3* gene binds to RORγt inhibiting its action. Also, STAT5 competitively

binds to *IL-17* along with STAT3, preventing expression [2,6]. These cells, however, are joined in their inhibition of T_H1 and T_H2 cells, by TGFβ as this turns off GATA3, *IL-4*, and *IL-5* among other genes. It also turns on *IL-9*, inducing T_H2 to T_H9 plasticity[6].

T_{reg} cells have a high affinity IL-2R due to IL-2's importance as a survival-growth factor. It abrogates IL-2 from the surrounding environment, and so very effectively inhibits T_H1 and T_H2 survival, but as IL-2 inhibits T_H17, this could be a fault in its regulatory function against T_H17[5]. T_{reg}s may also resort to plasticity as a form of regulation, as it has been recorded that supplying IL-23 to T_H2/T_H1 populations can induce T_H17, or the opposite direction with an appropriate cytokine can also be achieved. This is very difficult to do, but may be viable due to the instability of iT_{reg}s and so the lack of their ability to monitor these cells at all times[2,6,8]. In X-linked immunodeficiency polyendocrinopathy enteropathy (IPEX), *Foxp3* has a frameshift mutation, leading to fatal autoimmunity, usually by a T_H2 hyperactivity related condition. As Foxp3 functionality is a non-redundant requirement for T_{reg}s, this highlights the importance of T_H regulation to avoid autoimmunity and/or allergy[2,6,9].

These pathways all reveal a very stringently controlled system, and this is for a reason. In the collapse, or bypass of this system, abnormal activity of $CD4^+$ cells can have detrimental effects on the body, even to the point of fatality. Using the above example as a starting point, T_H2 dysregulation typically is associated with allergy. Their production of IL-5 recruits eosinophils, and IL-4 and IL-13 induce class switching to IgE[2]. These then bind to $F_c\varepsilon RI$ located on mast cells and basophils, activating them. This is called sensitisation and upon exposure to the allergen the immunoglobulin recognises, these cells degranulate releasing histamine and heparin[10,11].

Alternative macrophages accompany in this condition, and can release more cytokines exasperating the allergic situation. Such cytokines include IL-25, IL-33, TSLP and IL-9. Recruited eosinophils produce IL-25, promoting T_H2 function. This increases IgE levels and mucin production by goblet cell metaplasia for example. IL-33 acts in the same way. The allergenic role of IL-9 highlights the problem with dysfunctioning of T_{reg} cells, as T_H2 induction to T_H9 has been mentioned as a mechanism used for alleviation of T_H2 activity. IL-9R is present on basophils, and so their activation by IL-9 from T_H9 cells results in the expansion of the T_H2 population, and it also increases activity of eosinophils , due to the cytokines that activated basophils release[8,10].

A further example of how lack of regulation can lead to a T_H2 skew is the presence or absence of Vitamin D_3 and IL-10. Although producible by a great number of cells, both nT_{reg} and iT_{reg} cells are prime sources of IL-10, and in many cases are responsible for the induction of IL-10 production in T_H1 and T_H2 cells, so causing self-regulation. IL-10 is key to reducing IgE levels, and Vitamin D_3 is required for T_{reg} sustaining and so IL-10 maintenance[9]. Consistent exposure to the common particles (allergens) is required for the T_{reg} population to maintain the specific IL-10 production. A drop in this can lead to a drop in T_{reg}s and so a rise in T_H2[9]. In fact, even with T_{reg} cells, polymorphic *IL-10* promoters have been highlighted in the reduction of IL-10 mRNA and increase in proinflammatory mRNA in allergy[9].

However, despite this obvious role of T_H2 cells in allergies, and particularly in asthma, more cells direct the immunopathology than just T_H2. For example, T_H17 cytokines, such as IL-17, can be detected in metal allergies, atopic dermatitis and also in the sputum of non-eosinophilic asthma patients, along with neutrophils. When only T_H2 cells are detected, elevated eosinophil levels are present; with T_H17 only asthma, elevated neutrophils can be found but with both T_H2 and T_H17 cells present, extreme eosinophilia sets in. This seems illogical, as the co-presence allegedly promotes T_H2 function. Also, the method used to sensitise the patient seems to be important, as Langerhans cells in an ovalbumin sensitised (OVA) mouse model induce T_H17 cells. The resulting correlation between T_H17 cytokines and neutrophilic presence and activity reveal a prominent role of T_H17 in this particular asthma activation pathway[3].

The combination of antigen presentation with TSLP can be used to initiate T_H2 activation, and so this would also be usable in allergies. In an allergic situation, such as allergic asthma, if this is combined with dsRNA (highlighting viral presence) a very strong polarisation to T_H2 and T_H17 occurs. This therefore induces a very potent inflammatory response to this infection, and so viruses can be seen to aggravate such conditions[3]. T_H2 cells but also T_H1 and T_H17 cells, particularly in inflammatory allergic conditions, have all been detected and associated with this regulatory breakdown. This reinforces the statement that allergic conditions are not down to one cell's dysregulation, but that a complex involvement of many cell subsets is present[3].

It has been hypothesised that there could be plasticity from T_H17 to T_H2, as only half of the original T_H17 cells in some studies produce T_H17 cytokines in the allergic patient, and also chronic progression through various cells over the course of the condition is plausible[3,11]. Therefore, targeting purely the T_H2 cells may not prove effective, e.g. via corticosteroids, due to the presence and proliferation (that would result from a drop in T_H2) of other, inflammatory cells[3]. The inclusion of Vitamin D_3 with the allergen doses in immunotherapy seems to be promising, as this could require less allergen to be provided, lessening the risk of anaphylaxis, but also promoting IL-10 production and T_H suppression or polarisation away from T_H2[9].

Hypersensitivity doesn't only apply to the immediate form, as in allergy, but also immune cells may be directed against self antigens, as is the case in autoimmunity. Originally, T_H1 cells were designated the main protagonists, owing to their persistent presence with cytokines in experimental autoimmune encephalitis (EAE), rheumatoid arthritis, Crohn's disease and psoriasis sites. However, *IL-12*$^{-/-}$ genotypes provided no resistance to the conditions. In fact it made them worse. IL-12 is heterodimeric, composed of p35 and p40 subunits. IL-23 shares the p40 subunit, combined with p19, to make its structure. *IFN-γ*$^{-/-}$ and *p35*$^{-/-}$ had the same effect as total loss of IL-12, but *p40*$^{-/-}$, *p19*$^{-/-}$ and *IL-23*$^{-/-}$ all conferred resistance against these pro-inflammatory autoimmune conditions[1,3].

The focus on genes here is significant, due the strong role genetics has regarding autoimmune susceptibility. For example, dizygotic twins have a 5-6% chance of developing the same autoimmune disease. However, a pair of monozygotic twins have a 30-50% chance of having the same autoimmune hypersensitivities[11].

T_H17 cells are very potent in autoimmune inflammation, however the presence of T_H1 cells does signify at least some involvement, potentially in continuation. The co-expression of IFN-γ and IL-23 in chronic conditions further exemplifies this, but even more accurate is the rise in T_H1 cytokines after the fall in the instigating IL-17, promoting a hypothesis of complex systematic processes that go through cellular phases[4,5]. In various types of autoimmunity, auto-antibodies are seen to be involved, highlighting a potential role of T_H2 cells, but consistently the employment of inflammatory and cytotoxic cells by T_H1 and T_H17 cells is a signature feature.

The positive feedback loop between prostaglandin E_2 (PGE_2) from macrophages and IL-17 from T_H17 cells causes the proliferation and maintenance of potent T_H17 cytokines, including up-regulated IL-1β, IL-6, IL-17 and IL-22. In particular, IL-17 and IL-17F are seen to be non-redundant in autoimmunity[5]. Not only do the T_H17 cells produce these cytokines, but in autoimmunity the production of pro-inflammatory cytokines from other cells is induced, such as IL-23 and TNFα from Paneth cells in Crohn's Disease and Ankylosing Spondylitis. This is possibly due to up-regulated IL-23R in these conditions. The massive amounts of these cytokines being produced, and the fact that it encourages a positive feedback system, employing more macrophages, neutrophils and T_H17 cells reinforces the importance of T_H17 cytokines in autoimmunity[4,5].

Due to the expression of Aryl hydrocarbon receptor (AhR) on T_H17, the binding of aromatics, such as carbazole from cigarette smoke, can cause the exasperation of an already serious autoimmune condition. LPS can also aggravate autoimmune conditions, such as in Crohn's disease, as the secretion of pro-inflammatory IL-22 results from TLR activation by LPS[5,6].

High levels of iT_{reg} cells are detectible in autoimmunity, but they are unable to quell the proliferation and activity of the inflammatory T_H1 and T_H17 cells, despite releasing high levels of SOCS3 which inhibits IL-23. This is due to overwhelming levels of IL-6, TNFα and DC activation of cells. Even complete removal of TGFβ doesn't provide resistance, as although T_H17 cells are no longer present, there is now a lack of T_{reg}s. This allows severe hyperactivity of both T_H1 and T_H2 cells, resulting in 100% mortality in mouse models[1,5,6].

What is obvious from the study of this topic is that T_H cell dysregulation is not solely responsible for autoimmunity and allergy. Of course the breakdown of tolerance, and regulation are severally detrimental, but genetic susceptibility regarding MHC phenotypes, such as HLA-DR3 and HLA-DR4 in type 1 diabetes mellitus, must also be considered. Beyond this, environmental factors outside of the body's control, innate cell activity, APC activity, the characteristics of the cells to which the allergic or autoimmune condition is localised, and many other factors all play a role in the development of hypersensitive conditions.

The only true inference that can be taken from this information is that despite strong associations of certain cells with specific conditions, no one single cell is responsible for a condition. T_H2 are accompanied by T_H17 in allergies, and T_H17 by T_H1 and T_H2 in autoimmunities, just showing how the only common feature throughout all of these conditions is that they all involve immensely complex and potent processes, many cells and no simple answers.

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