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Abstract

The *respiratory syncytial virus* (RSV) represents the leading cause of viral bronchiolitis and pneumonia in children worldwide and is associated with high morbidity, hospitalisation rate and significant mortality rates. The immune response elicited by RSV is one of the main factors contributing to the pathogenesis of the disease. Two subsets of the cellular immune response, the T helper 17 cell (Th17) and the regulatory T cell (Treg), and more particularly the balance between these two subsets, might play a significant role in the pathogenesis of the RSV infection. The developmental pathways of Th17 and Treg cells are closely and reciprocally interconnected and plasticity has been demonstrated from Treg towards Th17. During an RSV infection the functions of both subsets are opposed to one another regarding viral clearance and clinical severity. Th17 and Treg cells offer a promising new view on the pathogenesis of an RSV infection and deserve further exploration.

Introduction

The *respiratory syncytial virus* (RSV) is the leading cause of viral bronchiolitis and pneumonia in children worldwide. Seventy percent of children are infected in their first year of life, reaching up to 100% in the second year of life (1). Most frequently, the clinical picture consists of mild upper respiratory tract symptoms, while up to 40% will present with lower respiratory tract infections, most frequently bronchiolitis (2). RSV infections are the most important reason for hospitalisation in children with respiratory symptoms, of whom 1-2% require transfer to an intensive care unit with an associated mortality rate up to 10% (1,2). Fortunately, mortality merely related to bronchiolitis is rare (0,13%) and in most fatal cases underlying cardiac, respiratory and/or immunological diseases are present (2,3).

RSV RNA encodes 11 proteins, including two non-structural proteins NS1 and NS2, a surface attachment glycoprotein G and a surface fusion glycoprotein F (4,5). Protein F mediates the fusion between the virus and the host cell membrane and as such promotes formation of syncytia, giving the virus its name (1). Protein G is responsible for binding cell surface glycosaminoglycans and is necessary for in vivo viral replication (4).

It is generally accepted that an RSV infection is a multifactorial process, including genetic constitution, environmental factors, lung epithelium-related factors and immunological responses. Disease severity has been correlated with specific viral genotypes: variability in the aforementioned G protein could have implications on the virulence of the individual virus. Another important factor is the genetic susceptibility of the host; several genetic markers that predispose to a more severe clinical picture have been identified including Tumour Necrosis Factor α (TNF-α) and RANTES. Finally, the age at infection, and thus lung maturity, is also seen as a parameter defining disease severity (6). All of these elements could have their role in defining the eventual polarisation and cytokine profile of the immune response following RSV infection.

RSV possesses the ability to avoid the normal immune memory resulting in the possibility to reinfect the patient with the same strain (1,7). Both innate and acquired immune responses are essential for an

effective viral clearance. The humoral response is important in the protective immunity against RSV infections as antibodies hamper the infection. However, once infection is established, an effective B cell response with efficient neutralising antibodies is absent (7). Therefore, RSV clearance is brought about predominantly by a cellular T cell response (2,7). Naive T cells will differentiate into specific T cell subsets depending on the presenting stimulus and the immunological environment. The balance between different T cell subsets sets the stage for the acquired immunological response. Initially, the induction of mainly T helper 2 (Th2) cells rather than T helper 1 (Th1) cells, deregulating the Th1/Th2 balance, was put forward as an explanatory theory to predict the severity of an RSV infection (1,8). However, more recently, evidence has been presented pointing to the role of two other T cell subsets determining the nature of the immunological response and therefore the severity of the RSV infection, namely the interleukin (IL) 17 producing T helper 17 cells (Th17) and regulatory T cells (Treg). The balance between Th17 and Treg cells has been suggested in the setting of several autoimmune diseases including rheumatoid arthritis, lupus and inflammatory bowel disease (9,10). Their importance will be this review's main emphasis.

Th1 cells, Th2 cells and RSV

In the 1960s, the development of a formalin inactivated RSV (FI-RSV) vaccine failed in human trials as it resulted in exacerbated disease and increased need for ventilatory support during subsequent natural RSV infection (11). As a predominant Th2 response was demonstrated in these cases, the imbalance between Th1 and Th2 cells became an important model to explain the differences in clinical severity in individual RSV infections (8). The most desirable T helper response following an RSV infection is of the Th1 type, due to its production of interferon-γ (IFN-γ), TNF-α and IL-2, thus activating cytotoxic T cells and natural killer (NK) cells leading to efficient viral clearance (1,12). When, however, a Th2 immune memory is generated following an infection or vaccination, a more pronounced and even detrimental inflammatory response will arise upon renewed contact with RSV, due to the secretion of IL-4 and IL-5 by Th2 cells. In addition, the Th1 and Th2 responses are known to downregulate each other's activation, as will be described below (13). For these reasons, a dominant Th2 response will result in an inefficient viral clearance and increased disease severity (1,8,12). It has been demonstrated

in mice that the T cell response is biased towards Th1 when primed by the RSV F protein, while the G protein primes towards the Th2 response (14,15). RSV's NS1 protein inhibits differentiation of Th1, Th2 (and Th17) cells. The NS2 protein inhibits differentiation of Th2 (and Th17) (16). Another skewing of the Th1/Th2 response is present when primary RSV infection occurs at the neonatal age: a Th2 rather than the appropriate Th1 response will develop upon reinfection, while this is not seen in adults (17).

Arguments for an imbalance in the Th1 and Th2 responses during severe RSV infections were indeed provided by some studies, demonstrating on the one hand a Th2 predominance with increased IL-4 concentrations in serum or nasopharyngeal aspirates of RSV infected children and on the other hand a decreased Th1 response with decreased IFN-γ concentrations in serum or nasopharyngeal aspirates (12,18-20). An increased IL-4/IFN-γ ratio correlated with an increased severity of the infection (12). However, the Th1/Th2 balance, as explanatory model for the pathogenesis of the RSV infection, is not absolute and has been contradicted by several human studies in which a Th2 predominance (21-25) and/or a decreased Th1 response (22,23,25) were not confirmed, implying that different mechanisms are involved in the immune-mediated events during severe RSV bronchiolitis.

Treg cells

Main characteristics

Treg cells are immunoregulating cells that express the transcription factor forkhead box P3 (Foxp3) (26). Their principal action is to maintain the immunological equilibrium, thus contributing to the body's homeostasis, by avoiding excessive effector T cell activation and tissue damage during induced immune responses against infections (27-29). The Treg cells exert this function by production of the inhibitory cytokines IL-10 and transforming growth factor β (TGF-β) (27,30,31), by interference with T cell survival through IL-2 depletion (27,32) and by secretion of molecules that directly eliminate effector cells (27) and inhibit antigen presenting cell (APC) maturation and functionality (27,31,33).

Treg cells during RSV infection

Shortly after RSV infection, Foxp3$^+$ Treg cells accumulate in the lungs and mediastinal lymph nodes in mice (34). This corresponds with a pronounced reduction of peripheral blood Treg cells in infants with a severe RSV infection, which could be explained by an increased recruitment to the lungs and lung-draining lymph nodes, increased apoptosis and/or increased plasticity to effector-like phenotypes (35).

The effects of depletion of Treg cells were described in several murine studies:

Firstly, a less efficient viral clearance due to a delayed recruitment of RSV specific CD8$^+$ cytotoxic cells to the lungs was demonstrated. This was accompanied by a delayed exacerbation and a slower recovery. However, no difference in the final viral clearance was seen post-infection as it was achieved completely in both the controls and, albeit slower, in the Treg depleted mice (34,36,37). Loebbermann *et al.* on the other hand, did describe a decrease in viral load when depleting Treg cells in mice (38). These findings can be explained by the fact that during an RSV infection, Treg cells are responsible for early recruitment of activated CD8$^+$ cytotoxic cells out of the draining lymph nodes to the lungs (37). When the Treg response is inhibited, delayed migration of the CD8$^+$ T cells out of the peripheral lymph nodes results in delayed viral clearance and increased disease severity (34). In conclusion, Treg cells by no means hinder the viral clearance of RSV and may even facilitate the process (34,36,37).

Secondly, a significant increase of the influx and persistence of CD4$^+$ and CD8$^+$ T cells - despite their delayed recruitment - as well as a greater influx of eosinophils was demonstrated in the airways of the Treg-depleted mice. Combined with an observed splenomegaly due to proliferation of CD4$^+$ and CD8$^+$ T cells, this suggests a systemic inflammatory response and an excessive RSV-specific T cell response in Treg depleted mice. In this setting, the abundant presence of CD8$^+$ T cells may surpass their contribution to effective viral clearance and may lead to tissue pathology and an increased disease severity, due to an increased, unrestrained production of TNF-α and IFN-γ by the CD8$^+$ T cells (34,39).

Thirdly, spontaneously developing Th2-type pathologies were described in Treg depleted mice as Treg cells not only control the immunological environment in order to avoid excessive inflammatory T cell responses, but also aid to limit inefficient Th2-type immune responses (39). In contrast to

depleting Treg cells, stimulating the Treg cells in function and numbers by using IL-2/anti-IL-2 immune complexes limits lung inflammation and disease severity without preventing viral clearance (38).

In IL-10 knockout mice, RSV induced greater disease severity, increased levels of proinflammatory cytokines and chemokines, increased lung pathology and an increase in the virus-specific IFN-γ-producing $CD8^+$ and $CD4^+$ T cells (40,41). IL-10 receptor (IL10R) blockade with anti-IL-10R antibodies resulted in a decreased number of Treg cells with a concomitant increase in the number of Th17 cells (41). In conclusion, production of IL-10 by Treg cells contributes to maintaining an adequate immune response during RSV infection.

Interestingly, a difference in the Treg response is observed when comparing primary to secondary RSV infections: during primary RSV infection Treg cells expand to maintain an immunological environment favouring viral clearance, while upon secondary infection, a diminished Treg response is observed. This was related to the increased disease severity in the setting of prior FI-RSV vaccination, while this relation was not seen upon secondary exposure to natural RSV infection (42). In a study by Wang *et al*. an asthmatic phenotype was induced by sensitizing mice to ovalbumin, resulting in an increased Th17 response and a defective Treg response leading to Th2-mediated airway inflammation. Upon primary RSV infection this imbalanced Th17/Treg response was reversed, while secondary RSV infection induced an acute asthma exacerbation by worsening the existing Th17/Treg imbalance (43).

Thus, during RSV infection Treg cells have proven their importance in ensuring efficient viral clearance by coordinating recruitment of $CD8^+$ cytotoxic T cells to the lungs, avoiding an excessive RSV-specific T cell response (both $CD4^+$ and $CD8^+$) and thus an excessive inflammatory response, limiting inefficient Th2-type immune responses and controlling the innate immune response by neutrophils and NK cells. Defective or suboptimal Treg function during RSV infection may cause immunopathology and hence a more severe clinical picture (44). While the role of the Treg cells as key resolvers of the lung inflammation caused by RSV infection has been established in mice, their role in human RSV-induced disease remains to be further defined (45).

Th17 cells

Main characteristics

Th17 cells are IL-17 producing, proinflammatory cells that express the transcription factor retinoic acid receptor-related orphan receptor γt (RORγt) (46,47). Their principal action is to stimulate inflammatory reactions and reinforce the acquired cellular immune response against extracellular bacteria, fungi and viruses (9,48). Due to the ubiquity of the IL-17 receptor on epithelial cells, endothelial cells, monocytes and macrophages, IL-17 induces a powerful proinflammatory response by stimulating secretion of proinflammatory molecules (46).

Th17 cells and RSV

Multiple studies were performed in mice to assess the role of Th17 cells during RSV infections. RSV is capable of inducing a Th17 response through activation of complement factor C3a and tachykinins (49). Neutralising IL-17 in RSV infected mice led to the reduction of mucus production, inflammation and viral load, an increased number of $CD8^+$ cells and a reduction of Th2 cytokines (50). Moreover, induction of IL-17 by RSV appears to attenuate suppression of Th17 development by IL-27 (51).

Children suffering from a severe RSV infection showed increased IL-17 levels in their tracheal aspirates, as well as elevated plasma concentrations of IL-17 and IL-8 (50,52,53). When comparing the IL-17 concentrations in non-ventilated versus ventilated RSV-infected children, it was demonstrated that the IL-17 concentrations in plasma were higher in the non-ventilated group while a significant difference was not present in the nasopharyngeal aspirates (52,54). The nasopharyngeal concentrations of other proinflammatory cytokines such as IL-1α and IL-6, however, were higher in ventilated infants. Another difference between these two populations was demonstrated by measuring the nasopharyngeal IL-17 concentrations at discharge: in the non-ventilated infants these were significantly higher than at admission, while this was not seen in the ventilated group. The explanation for this seemingly paradoxical reaction, i.e. a higher IL-17 level in moderately ill (non-ventilated) when compared to severely ill (ventilated) RSV-infected children is still controversial (54). In line with these results, it has

previously been demonstrated that a greater proinflammatory response seen in nasal samples was associated with a less severe bronchiolitis, while a less pronounced inflammatory response was found in critically ill patients (52).

A role for IL-17 is thus clearly demonstrated in RSV infections and four underlying mechanisms resulting in severe RSV infections are proposed. Firstly, IL-17 will cause exaggerated mucus production (50,55). Secondly, IL-17 is thought to enhance Th2 cytokine production in a Th2 skewed environment (50). IL-13, a proinflammatory cytokine primarily secreted by Th2 cells, is a known critical mediator of airway hyperresponsiveness, directly modulating airway epithelial mucus production, influencing eosinophil migration into the lungs, and possibly also acting as a direct smooth muscle spasmogen (56). In contrast, IL-17 has no direct effect on airway hyperresponsiveness, which is solely Th2-mediated (50). In this context, a number of studies showed that RSV-infected mice simultaneously produced IL-17 and IL-13 suggesting that the Th17 response is concomitant with the Th2 response (50,55,56). Thirdly, IL-17 has been associated with increased neutrophilic infiltration in the lungs. This effect is brought forth by enhancing the production of CXC chemokines by stromal cells, such as IL-8 (50). Fourthly, IL-17 seems to diminish effector $CD8^+$ T cell responses through negative regulation of T-bet and Eomes, two transcription factors that regulate the $CD8^+$ cytotoxic T cell effector functions, thus diminishing viral clearance (50,55).

Mechanisms controlling T cell differentiation

The transcription factors and various cytokines involved in determining T cell differentiation are depicted schematically in Figure 1.

Th1 subset

The critical cytokines for Th1 differentiation are IL-12 (produced by APCs and macrophages) and IFN-γ (produced by NK cells), which activate the transcription factors Signal Transducer and Activator of Transcription (STAT) 1 and STAT4 (57). STAT1 further stimulates T-bet, the characteristic transcription factor of the Th1 lineage. Together, STAT4 and T-bet ensure Th1 differentiation and create

a positive feedback loop through IFN-γ production (57,58). Moreover, T-bet inhibits GATA3 and RORγt functionality, thus impeding Th2 and Th17 differentiation and favouring Th1 differentiation. In a later stage IL-18 further stimulates IFN-γ production, enhancing differentiation towards the Th1 subset (57). Both TGF-β and IL-6 inhibit Th1 differentiation by limiting the expression of T-bet and STAT1, respectively (57-59).

Th2 subset

The critical cytokines for Th2 differentiation are IL-4 and IL-2. IL-4 is initially produced by naïve $CD4^+$ T cells and induces the transcription factor STAT6, which in turn induces GATA3, the characteristic transcription factor for the Th2 lineage (57,60). GATA3 and STAT6 enhance Th2 cytokine production. In the presence of IL-6 more IL-4 will be available due to increased production by naïve T cells. IL-2 activates an IL-4 independent pathway through STAT5 and is essential for the production of IL-4 by Th2 cells. Moreover, GATA3 inhibits Th1 differentiation by interacting with T-bet and downregulating STAT4 (57). TGF-β inhibits Th2 differentiation by limiting expression of GATA3, independently of STAT6 (57,61).

Treg subset

Differentiation towards the Treg subset depends on the presence of TGF-β in absence of IL-6 (62,63). The essential role for TGF-β in the differentiation of Treg cells is exerted through induction of the STAT5 transcription factor (63,64). IL-2 and retinoic acid further reinforce differentiation towards the iTreg subset (65). IL-2 achieves this by inducing the transcription factor STAT5, which in turn will enhance Foxp3 expression, and retinoic acid by promoting TGF-β signalling and Foxp3 promoter activity while inhibiting Th17 differentiation by blocking IL-6 signalling (47,64). IL-10 also plays a part in promoting differentiation towards the Treg subset (66).

Th17 subset

Mice

The critical cytokines for Th17 differentiation are IL-6 and TGF-β. Together, they induce RORγt, the characteristic transcription factor of the Th17 lineage, in a STAT3 dependent manner (9,46). Moreover, in the presence of IL-6, Th17 cells can secrete TGF-β in an autocrine manner thus contributing to a stable and maintained Th17 response (67). The absolute requirement for TGF-β is attributable to the inhibition of Th1 cell differentiation, since Th1 cells strongly inhibit Th17 differentiation in the absence of TGF-β (62).

IL-21 is capable of enhancing Th17 differentiation and proliferation as well, resulting in a positive feedback loop since Th17 is the major producer of IL-21 (46,62). On the other hand, IL-21 is known to supresses Foxp3 (62). The cytokines IL-1β and TNF-α play an accessory role, as they further augment differentiation of the Th17 cells (68). More importantly, IL-23 is essential for terminal differentiation of the Th17 subset and exerts this function in a STAT3 dependent manner (62,69). By upregulating its own receptor on Th17 cells, yet another positive feedback mechanism is ensured (62).

Negative control over Th17 differentiation is regulated through four distinct mechanisms: IL-27 and IFN-γ through STAT1 activation (9,46,51,70,71); IL-2 and IL-4 through STAT5 activation (72,73); IL-13 through stimulation of IL-10 production by Th17 cells, leading to IL-6 downregulation and thus diminished IL-17 production (74); loss of the inhibition of RORγt by Foxp3 when IL-6 is present (75,76). The balance between Foxp3 and RORγt is therefore a very important factor in the Th17/iTreg equilibrium.

Humans

The exact mechanisms involved in the differentiation into Th17 cells are less clear in humans. In contrast to the situation in mice, the combination of IL-6 and TGF-β alone does not suffice for human Th17 differentiation; the cytokines IL-1β, IL-21 and TNF-α are also essential (77-79). Whether TGF-β is necessary for human Th17 differentiation, remains a matter of debate (80).

Influence of RSV on T cell differentiation

RSV stimulates the secretion of a multitude of cytokines: IL-10 and IL-6 by DCs (81); IL-1β by macrophages (82); IL-6, IL-23 and TGF-β by epithelial cells (83); TNF-α by neutrophils (6). The secretion of these particular cytokines suggests favouring of Th17 differentiation. RSV-infected human bronchial epithelial cells (HBEC) enhance the expression of the inflammatory transcription factor NF-κB and release multiple chemokines (intercellular adhesion molecule-1, IL-6, IL-8 and RANTES) leading to recruitment and differentiation of neutrophils, eosinophils and T helper cells. Differentiation into Th17 cells is stimulated, while the differentiation into Treg cells is inhibited (84). Moreover, RSV is able to induce a Th2-like effector profile of the Treg cells by inducing GATA-3. This results in the loss of the Treg cell's normal suppressive function and causes allergic airway disease (85). The exact mechanisms by which RSV exerts its effect on T cell differentiation is yet to be clarified. Recent studies indicate potential roles for the autophagy-associated protein Map1-LC3b in Th17 differentiation and the H3K4 histone methyltransferase SMYD3 in Treg differentiation (86,87).

Plasticity

The plasticity of T cells ensures the adaptability of the immune response to the local environment. This is emphasised by the discovery that Treg cells can differentiate into Th17 cells, particularly when exogenous IL-1β, IL-23 or IL-21 is administered *in vitro* (88). This plasticity is further demonstrated by the existence of $Foxp3^+$ $ROR\gamma t^+$ $CD4^+$ T cells in which characteristics of both Treg cells ($Foxp3^+$) and Th17 cells ($ROR\gamma t^+$) can be found. There seems to be considerably more plasticity in the Th17 lineage than in the Th1 and Th2 lineages which are resistant to further differentiation due to epigenetic modifications of the associated gene loci (28). At least 25% of Th17 cells once expressed Foxp3. This could point out the ability of $Foxp3^+$ Treg cells to differentiate into Th17 cells when the appropriate proinflammatory cytokines are present (76).

Plasmacytoïd DCs are able to induce IL-17 secretion from $Foxp3^+$ Treg cells associated with an inhibition of Treg suppressive activities (89). Human Treg cells are largely resistant to IL-6 and differentiate to the Th17 lineage in an IL-1-dependent manner. Therefore, infiltrating Treg cells at sites of infection where IL-1β is highly expressed may not necessarily exert a suppressive function, but might

instead participate in the inflammatory reaction against the inciting pathogen through conversion to the Th17 lineage (28).

The reverse, i.e. differentiation of Th17 cells into Treg cells, has not yet been described (65). In turn, Th17 cells also display phenotypic instability as they are able to convert into Th1 cells in a STAT4- and T-bet-dependent manner. This might be important after the initial Th17 mediated response to successfully clear the excessive inflammation (29,65). In conclusion, the human regulation of the T cell balance is a very dynamic process. Understanding this delicate equilibrium could help create new opportunities in the treatment of infections by viruses, such as RSV (75).

Th17/Treg balance in RSV and its clinical relevance

As shown above, the developmental pathways of Th17 and Treg cells are closely and reciprocally interconnected. In addition, plasticity from the Treg subset to the Th17 subset has been demonstrated. These findings suggest a balance between both cell types. Moreover, the functions of both cells can be regarded as opposed to one another: Th17 cells promote inflammation which can even result in auto-immunity, Treg cells attempt to control the inflammatory responses and maintain self-tolerance. In this context the Th17/Treg balance has proved its potential in explaining the pathogenesis of several inflammatory and autoimmune diseases such as multiple sclerosis, rheumatoid arthritis and inflammatory bowel disease (9,10).

It has likewise been demonstrated that this balance is implicated in the pathogenesis of an RSV infection. The Treg and Th17 subsets have distinct and opposing features in this context, as demonstrated by their effects on viral clearance, clinical severity and Th2-oriented immune response (see Table 1). Specifically, the Treg immune response will ensure an efficient viral clearance and inhibition of the Th2-oriented immune response resulting in a less severe clinical picture (34,36-39). Although evidence is less conclusive than is the case for the Treg immune response, the Th17 immune response is thought to hamper an efficient viral clearance and further enhance a Th2 immune response resulting in a more severe clinical picture (50). Moreover, the $CD8^+$ T cellular response during RSV infection seems to be counteracted by Th17 cells through IL-17 while being stimulated by Treg cells through promoting early influx of $CD8^+$ T cells to the lungs, therefore resulting in efficient viral clearance (37,50,55). It is also

important to note the opposing effect of IL-10, i.e. inhibitory on Th17 cells and stimulatory on Treg cells (41,74).

In the 1960s, the FI-RSV vaccine led to exacerbated disease and increased need for ventilatory support during subsequent natural RSV infection. Administering this vaccine to mice resulted in a complete absence of Treg cells in the airways, enhanced lung inflammation and augmented clinical severity upon RSV infection. Moreover, increasing the number of Treg cells in the airways by selective chemoattraction with CCL17/22 reversed the enhanced lung inflammation and weight loss (11). An increased severity of RSV disease after FI-RSV vaccination was associated with a Th17-like memory response (90).

As described above, Qin *et al.* demonstrated that human bronchial epithelial cells, when infected by RSV, stimulate differentiation into Th17 cells, while inhibiting differentiation into Treg cells (84). Li *et al.* determined the presence of Th17 and Treg on peripheral blood, as well as the cytokine concentrations of IL-10, TGF-β and IL-17 on peripheral blood plasma in a population of 33 children suffering from RSV bronchiolitis. The percentage of Treg cells and the concentrations of IL-10 and TGF-β were significantly lower in this population, compared to children suffering from a non-RSV pneumonia and healthy controls. Meanwhile, the percentage of Th17 cells and the concentration of IL-17 were significantly higher, compared to children suffering from a non-RSV pneumonia and healthy controls (91). When Galectin-9, an indirect inhibitor of Th1 and Th17 responses, was administered to RSV-infected mice, the viral load decreased, mucus production was inhibited and lung pathology diminished: these effects were achieved by inhibition of the Th17 response and induction of the Treg response (92). These studies show that an imbalance between Treg cells and Th17 cells might be an important pathogenic factor determining clinical severity of an RSV infection.

Prevention

Because of the failure of the FI-RSV vaccine, only live attenuated RSV or RSV proteins expressed in a live virus vector have, so far, been considered safe for testing in RSV naïve children (93). Vaccines currently under investigation should promote protective and efficient immunity without adverse effects, prevent the Th2 immune response demonstrated upon FI-RSV vaccination and promote viral clearance

before disease establishment (94). However, up to now, no efficient vaccine has been licensed (93). Several studies are ongoing to evaluate multiple approaches in vaccine development. An overview of their progress in research is provided by PATH (95). For example, DNA vaccines based on the gene coding for RSV's G protein have been able to balance the production of Th1/Th2 cytokines in the lungs of mice during RSV infection, successfully evading the Th2 memory response. Another approach using the bacillus Calmette-Guérin bacteria (BCG) modified to express RSV's N and M2-1 proteins was able to induce the beneficial Th1 response and resulted in a decrease of pulmonary inflammation and reduced viral loads. As a last example, a subunit vaccine using RSV's F protein was able to elicit a Th1 response in mice as well (94).

Until an effective RSV vaccine will be licensed, our best preventive alternative consists of passive immunisation with antibodies, of which palivizumab is the best known example (96).

Concluding remarks

We can conclude that a balance between the Th17 and Treg cell subsets could play an important role in the pathogenesis of an RSV infection. A better understanding of the T cell subset profile during RSV infections, with particular interest in the balance between Th17 and Treg, their cytokines and transcription factors, is thus desirable. The cytokines involved in the differentiation of and the interaction between the different subsets should be a main point of interest. Influencing the leading cytokine profiles could eventually allow to modify the balance between the different T cell subsets in such a way, that it would be possible to predict, diagnose and treat each patient according to his or her individual immunological response to RSV.

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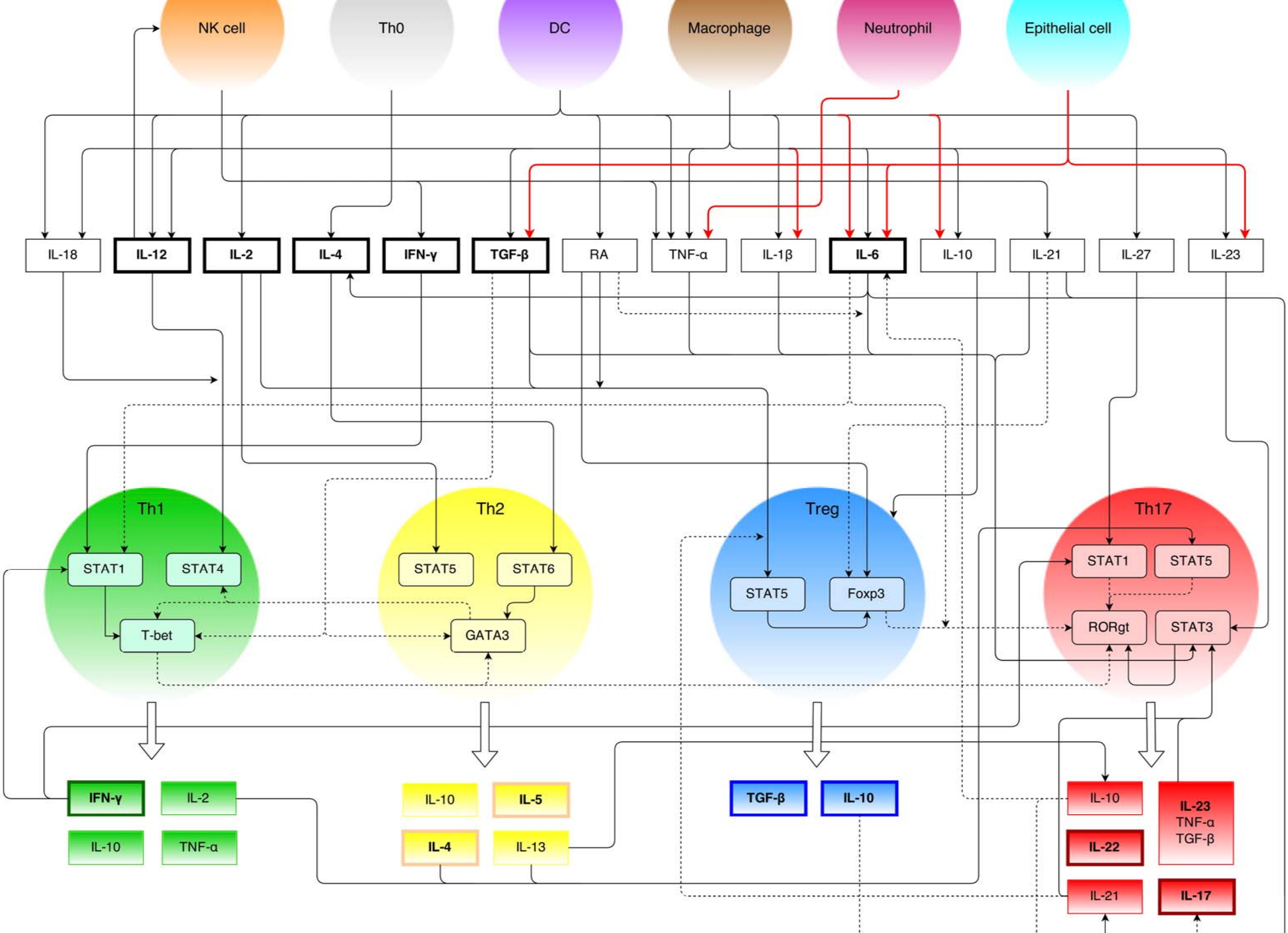
Figure legends

Figure 1. Differentiation of the Th1, Th2, iTreg and Th17 subsets

Full arrow: stimulation. Dashed arrow: inhibition. Bold: major cytokines. The red arrows depicts cytokines of which production is increased in the presence of RSV.

APC = Antigen Presenting Cell. Foxp3 = Forkhead Box P3. IFN-γ = Interferon-γ. IL = Interleukin. iTreg = inducible Regulatory T Cell. RA = Retinoic Acid. RORγt = Retinoic acid receptor-related Orphan Receptor γt. STAT = Signal Transducer and Activator of Transcription. TGF-β = Transforming Growth Factor-β. Th = T helper cell. TNF-α = Tumour Necrosis Factor-α.


NK cell
Th0
DC
Macrophage
Neutrophil
Epithelial cell
IL-18
IL-12
IL-2
IL-4
IFN-γ
TGF-β
RA
TNF-α
IL-1β
IL-6
IL-10
IL-21
IL-27
IL-23
Th1
STAT1
STAT4
T-bet
Th2
STAT5
STAT6
GATA3
Treg
STAT5
Foxp3
Th17
STAT1
STAT5
RORgt
STAT3
IFN-γ
IL-2
IL-10
TNF-α
IL-10
IL-5
IL-4
IL-13
TGF-β
IL-10
IL-10
IL-22
IL-21
IL-23
TNF-α
TGF-β
IL-17

Tables

Table 1: Effects of Treg and Th17 cells in RSV infections

Effects of Treg cells in RSV infections	Effects of Th17 cells in RSV infections
Ensuring efficient viral clearance by coordinating recruitment of $CD8^+$ cytotoxic T cells to the lungs	Diminished viral clearance through diminished effector $CD8^+$ T cell responses
Limiting inefficient Th2-type immune responses	Enhanced Th2 cytokine production in a Th2-skewed environment
Controlling the innate immune response by neutrophils and NK cells	Increased neutrophilic infiltration in the lungs
Avoiding excessive RSV-specific T cell responses (both $CD4^+$ and $CD8^+$)	Exaggerated mucus production