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Local elevation of CCL22: A new trend in immunotherapy (skin model)

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Abstract

Many evidences supported the suggestion that one of the reasons for the failure of immunosuppressant like Corticosteroides, Calcinurine inhibitors and VitD3 in reestablishing skin immune tolerance is relying on inhibition of CCL22 expression from skin dendritic cells. Inhibition of CCL22 decreases CD4+ CD25+ FoxP3+ regulatory T cells homing to macular area and reduces the suppression capacity of these cells that make a sort of an imbalance between effector and regulatory T cells. Addition of CCL22 into the skin lesion from external sources could change the ratio between effector and regulatory T cells which dramatically alter immune system and reestablish immune tolerance. This action can't be established by the later immunosuppressant (e.g. corticosteroids and calcinurine inhibitors) alone which give CCL22 an important role in the treatment of skin autoimmune and graft rejection diseases.

Keywords: CCL22; Langerhans dendritic cells; Corticosteroids; Calcineurine inhibitors autoimmune; Immunomodulation

1. Introduction

CCL22 Chemokine is one of a group of heparin binding proteins that induce migration and extravasation of chronically activated Th2 cells into the skin. They produce their action through binding to G protein-coupled receptor (CCR4) which in turn increase intracellular Ca^{++} mobilization that affect cytoskeleton-induced movement and increase the affinity of targeted cells to adhesion molecules [1]. The adhesion molecules E-selectin and intracellular adhesion molecule 1 (ICAM-1) are greatly upregulated in the dermal post capillary venules by cytokines released by skin resident antigen presenting cells like Langerhans dendritic cells. Langerhans dendritic cells can also produce activated T cell chemokines such as CCL22 on local endothelium which resulted in recruitment of cutaneous leukocyte antigenpositive (CLA+)and chemokine receptor 4 positive (CCR4+)CD4+ CD25+ FoxP3+ regulatory T cells in antigen non specific manner. In the skin, regulatory T cells encounter their cognate antigen specific Langerhans dendritic cells. So, they become activated and expanded to suppress activation and proliferation of effector T cells. This pool of suppressive T cells is prohibited in skin autoimmune diseases treated by Corticosteroides, Calcineurin inhibitors and VitD3 because they induce IL-10 expression which in turn inhibit CCL22 production by skin dendritic cells like Langerhans dendritic cells.

2. Macrophage derived chemokine (MDC)

CCL22 (Chemokine ligand 22) or Macrophage Derived Chemokine "MDC" was discovered in macrophages but it is also produced by activated B lymphocytes and dendritic cells [2]. It was also called "Stimulated T cell Chemotactic Protein-1"

Abbreviations: Tregs, regulatory T cells; CTL, cytotoxic T lymphocytes.

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(STCP-1) [3] and ABCD-1 (activated B cell-derived chemokine-1) or DC/B- CK (DC and B cell-derived chemokine) in mice [2,4]. In human, CCL22 gene is found in a mini cluster in chromosome 16q13 near gene of CCL17 (or, thymus and activation-regulated chemokine: TARC) and their Chemokines have homeostatic and inflammatory activity [5]. CCL22 and CCL17 bind the same receptor (CCR4) and induce Ca^{++} mobilization and chemo-attracting action on Th2 lymphocytes [6] with a preferential effect on CD4+ regulatory T lymphocytes [7].

CCL22 is constitutively expressed in dendritic cells, B cells, and macrophages whereas NK cells, monocytes, and CD4 lymphocytes express CCL22 after appropriate stimulation [8]. Langerhans dendritic cells, which are skin-epidermis populated immature dendritic cells, can also express CCL22 but only after maturation, however in mice it becomes the most abundantly expressed gene [4,9]. Although it is difficult to extrapolate these results to human, but langerhans dendritic cells had already taken a great consideration as homeostatic dendritic cells in human.

3. Langerhans dendritic cells and immune homeostasis in the skin

When langerhans cells encounter antigen in the epidermis, they drain to skin regional lymph node in response to CCL21 due to CCR7/CCL21 interaction. In lymph node, langerhans Dendritic cells present antigen to naïve T cells to become effector memory T Cells that express CLA (Cutaneus leukocytes Antigen) and CCR4 (the receptor for CCL22) beside the central memory T cells which reside in skin lymphoid organ and express E-selectin (adhesion molecule) and CCR7(the receptor for CCL21 and CCL19) Fig. 1. However, there are another pathways for trafficking Langerhans dendritic cells at steady state [10]. Upon second antigen encounter, effector memory T Cells home to skin in antigen nonspecific manner from regional skin lymph node through chemokine mediated homing. On the other hand; langerhans cells expand also long lived resident effector memory T cells [11].

While immunogenic action of langerhans cells had thoroughly been illustrated, their tolerogenic action is still being in its infancy stage. Ralph. M. Steinman shared the Nobel Prize in 2011 for his work on dendritic cells and their role in immunity. He uncovered the role of dendritic cells in peripheral tolerance by applying antigen in absence of danger signal [12] the work that gave the second wing for dendritic cells and opened the door to know their role in homeostasis of the immune system.

Julien Seneschal et al. (2012) illustrated the role of langerhans cells in maintaining immune homeostasis of the skin. The results emphasize that langerhans cells induce the proliferation of CD^{4+} $CD^{25+}FoxP^{3+}CD127^{-}$ skin resident regulatory T cells which located in the epidermis. These cells suppress

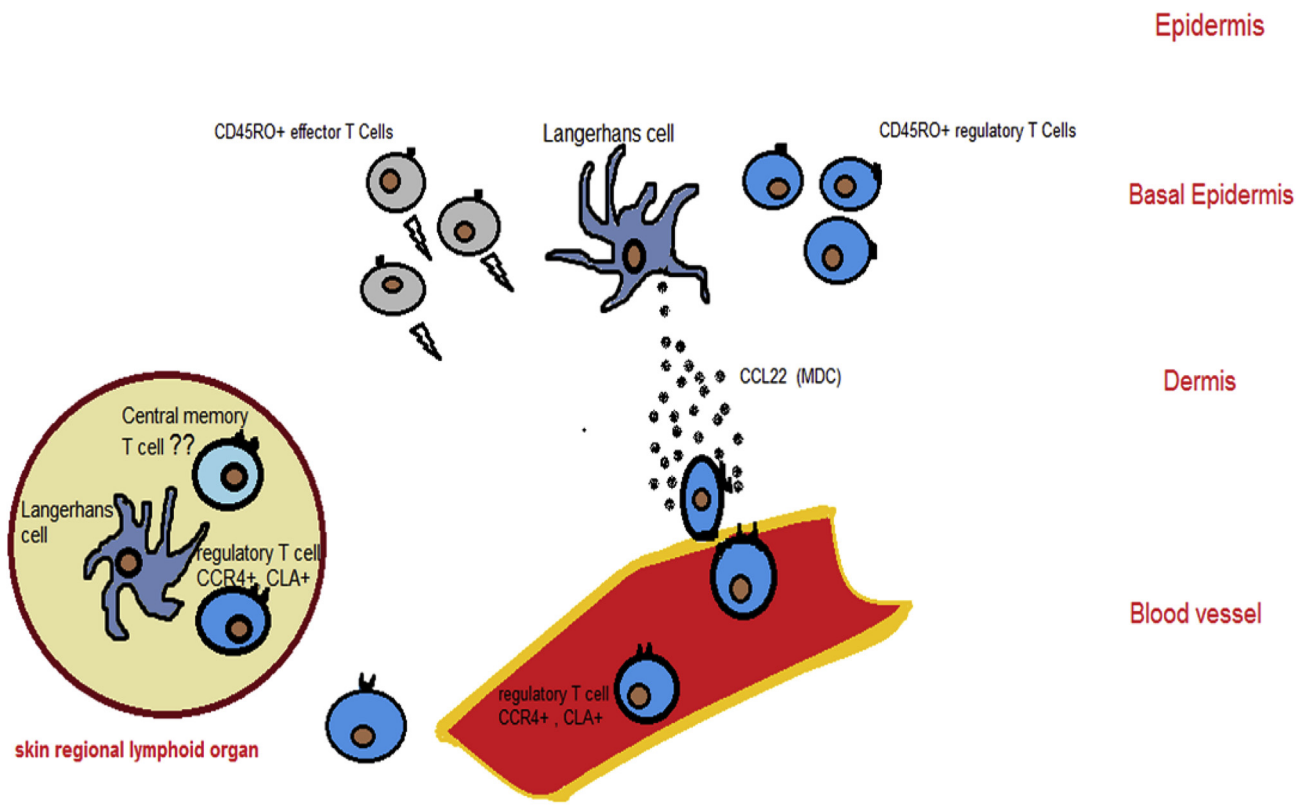

Fig. 1. Tolerogenic Langerhans dendritic cells and Regulatory T cells interaction in skin. This figure suggests that there are two sources of Tregs that populate the skin. One of them are resident in the skin and share the general marker (CD45RO+) with skin resident effector T cells. The second are the naïve T cells that differentiated into Tregs in the regional lymph nodes by maturation signal from dendritic cells drained from skin. The second group of Tregs with general marker (CCR4+, CLA) was suggested not to reach properly the organ specific autoimmune macular area in the skin under corticosteroids, calcineurin inhibitors and Vitamin D3 treatment because both of them suppress CCL22 production.

autoreactive effector memory T cells by cell to cell contact. However, langerhans dendritic cells induce also expansion of skin resident-effector memory T cells when they are co-cultured with *Candida albicans.* So, Julien et al. suggested that under steady state condition langerhans cells can keep peripheral tolerance but they can also activate effector cells against danger signals [13]. But, although many researches were carried out in this field, It is remain to know in accurate how to constantly programming tolerogenic langerhans dendritic cells.

4. Regulatory T cells populations in the skin

In the skin, there are skin resident memory T cells with general marker (CLA^+,$CD45O^+$,$CCR4^+$) 20 times more than in blood [13,14], resident memory ($CD4^+CD25^+FOXP3^+$ $CD127^-$) regulatory T cells constitute 5% of these cells [15]. They are antigen specific and expanded by Langerhans dendritic cells which occur side by side with skin resident $CD45O^+$ effector memory cells [13]. Beside skin resident memory T cells, skin dendritic cells specially langerhans cells present antigen to naïve T cells in draining lymph nodes. Activated T cells resulted from antigen presentation got either effector resident T cell markers ($CCR4^+$, CLA^+) or of central memory T cell markers (CCR7, L-selectin) [11] Fig. 1. Other type of Tregs are raised up in thymus by the process of negative selection, in which T cells that recognize self-antigens by T Cell Receptor with high affinity either died or selected to survive as Tregs. This type of Tregs is called natural Tregs and they are part of central tolerance, however some T cells escape this process and may cause autoimmunity [16].

It was suggested that loss of peripheral tolerance in the skin occurred in two steps: first, the induction phase in which the effector-autoreactive T cells were stimulated and expanded by $CD4^+$ T cells. These cells circulate in the blood but with no symptoms of autoimmunity whereas in the effector phase the skin microenvironment became favor for escaping peripheral tolerance [17] as in autoimmune microenvironment of non-segmental vitiligo lesions in which down regulation of CCL22 expression causes reduction in skin homing by functional Tregs and resulted in imbalance between effector and regulatory mechanisms [18].

According to their sources, the Tregs are either natural Tregs or inducible Tregs. They both are $FoxP3^+$ but were differentiated by neuropilin-1 (Nrp-1), which is selectively expressed on the surface of natural Tregs [19].

5. Major factors that regulate CCL22

Dendritic cells sense the antigens through various Pattern recognition receptors (PRR) expressed by dendritic cells. In response to this interaction, many cytokines and chemokines are secreted. In 2001 Vulcano M. et al. revealed the great role played by dendritic cells as the main source of CCL22 and stated different factors that regulate their expression summarized in Fig. 2. However in general, CCL22 is up regulated by cyclic AMP- elevating agents.

Among these factors:

5.1. Corticosteroids

Corticosteroids are immunosuppressant and anti-inflammatory compounds have variable effects on immune

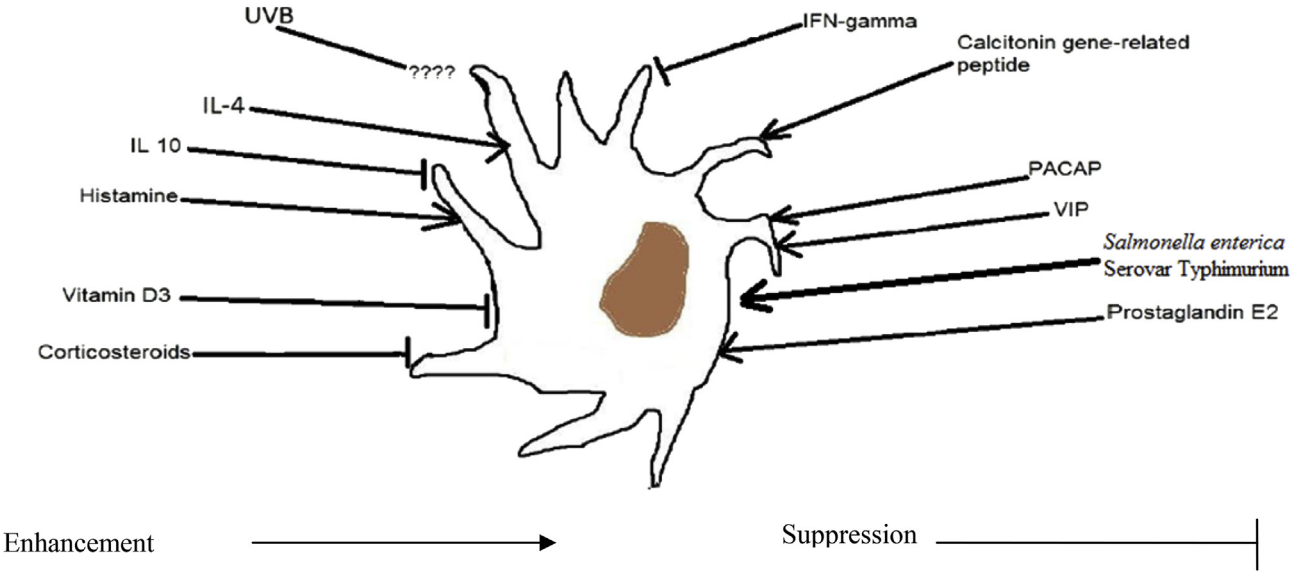

Fig. 2. Different factors regulate CCL22 or MDC (macrophage derived chemokine) expression. They include *Salmonella enterica* Serovar Typhimurium infected dendritic induces 31-and 150-fold increase in production of CCL22 within 2 and 6 h post infection. They contain also Cytokines; IL-4, IFN-gamma beside IL-10, Neuropeptides; Calcitonin gene related peptide, PACAP: pituitary adenylate cyclase activating polypeptide and VIP: Vasoactive Intestinal Peptide. Inflammatory mediator: Prostaglandin E2 and histamine. In general, all factors that increase cyclic AMP up-regulate MDC. Beside, Immunosuppressive agents like corticosteroids and vitamin D3. While, UV effect on CCL22 production is a paradox.

cells. They suppress immunity by modifying immune cells' responses to antigens.

There are many evidences for the role corticosteroids in suppression of CCL22 production by dendritic cells despite of their ability to proliferate Th2 cells and regulatory T cells. For example: Dexamethason down regulates CD83, IL-12 [20] in dendritic cells and induce production of IL-10 in T cells [21] that inhibits their ability to produce CCL22. Dexamethason also modify langerhans dendritic cells to up-regulate TGF-beta and expand dermal regulatory T CellsFOXP^{3+}CD^{25+} [22]. Beside Dexamethason; Tacrolimus and Cyclosporine also inhibit CCL22 production by Dendritic cells [20].

5.2. Ultra violet B

Different immunomodulation effects of UVB on keratinocytes were reviewed by (Brian Berman and Clay J. Cockerell, 2013). They characterize the membrane photo damage and UVB phospholipids interaction that modify keratinocytes to produce IL-10 and TNF-Alfa, which in turn generate tolerogenic dendritic cells that expand TGF-beta producing-CD^{25+} FoxP^{3+}regulatory T cells [23].

Langerhans dendritic cells upon exposure to UVB became unable to sensitize the body against selective antigen due to DNA damage that modifies langerhans cells to have immunosuppressive properties [24].

However, the effect of UV on the expression of CCL22 is a paradox. While it may down regulate CCL22 indirectly through IL-10 (produced by Tregs or keratinocytes), it may causes CCL22 up regulation through IL-4, prostaglandin E2, calcitonin gene related peptide, a melanocytes stimulating hormone and platelet activating factor [20].

5.3. Vitamin D3

Vit.D3 differentiates dendritic cells to develop either TGF-b secreting Foxp^{3+} Tregs or IL-10 secreting Tregs. While Vit.D3 modifies dendritic cells to expand IL10-producing Foxp^{3+} TR^{+} cells, it also modifies langerhans dendritic cells to expand TGF-beta producing FoxP^{3+} regulatory T cells* [25]. Peleen et al. in 2011, reviewed different roles played by Vit. D on dendritic cells, monocytes, NK cells, T cells, NKT cells and B cells in peripheral immune system. Like UVB, vitamin D3 down regulates different markers on dendritic cells (CD86, CD80, and MHCII), it also inhibits IL-12 production and up-regulates expression of IL-10 and TNF-Alfa [26].

Vitamin D3 reduces production of CCL22 from LPS induced−dendritic cell maturation indirectly through production of IL-10 which is known as a suppressor of CCL22 expression [20].

5.4. Salmonella enterica Serovar Typhimurium BRD509

In vitro studies of gene expression by bone marrow derived dendritic cells showed that dendritic cells infected byBRD509*Salmonella* induces production of CCL22 by 31- and 150-fold within 2 and 6 h post infection [27]. This mode of expression could be tested in recruiting (CD^{4+} CD^{25+} FoxP^{3+}) Tregs and others sub types *in vitro* and *in vivo* so as to evaluate their capacity for recruiting such type of regulatory T cells and also to further investigate Salmonella's genome and proteome to specify the antigens that induce this maximum expression of CCL22.

6. Model for study: non-segmental vitiligo

In vitiligo, the melanocytes antigens are carried by langerhans dendritic cells and presented to naïve T cells in the draining lymph nodes. The circulating cutaneous lymphocyte antigen (CLA)-positive T cells are circulating memory cells in the blood and have T cell receptors (TCRs) specific to antigens of melanocytes previously encountered in the lymph nodes such as: A2-melanA tetramere + CTLs isolated from vitiligo patients expressed high levels of CLA [17].

The immunohistochemistry showed infiltration of CD3+ T cells in dermis and few in the epidermis. The double staining of vitiligo biopsies by CD3+ and CD4+ showed that most of CD3+ cells are also CD4+. Whereas, CD3+/CD8+ are present in leading edge and lesional vitiligo skin and showing a mixture of CD8+ and CD4+ T cells beside the appearance of Th17 and Th1. However, double staining by CD3+ and FoxP3+ showed a significantly reduced percentage of Tregs among infiltrating T cells in non-lesional, perilesional and lesional vitiligo skin compared to normal skin from healthy adults [28].

In vitiligo perilesional skin, the increase in CD8+ T cells was not accompanied by increase in Tregs (called Tregs pausing). This significant reduction in Tregs is associated with marked reduction in CCL22 expression by 43% in vitiligo lesions comparing to controls, whereas the mode of expression of CCL17 and CCL1 was normal which lead to suggestion that a reduction in CCL22 expression is primarily responsible for impaired homing of Tregs. Whereas functional Tregs are abundant in the circulation [18]. So this support the hypothesis looking at autoimmune diseases as an increase in CTL comparing to Tregs and failure of Tregs to control CTL so as to keep “peripheral tolerance”.

7. Discussion

The commonly used immunosuppressant like Glucocorticoids and Vitamin D3 can modify dendritic cells to expand regulatory T cells which keep auto-reactive T cells in check. However, they suppress CCL22 (the major cytokine responsible for recruitment of regulatory T cells with CCR4+, CLA+ markers) to the affected area. Autoimmune diseases like Vitiligo have poor lesional specific expression of CCL22 and so lower regulatory T cells infiltrating the affected area than normal healthy skin. Theoretically, if we increase expression of CCL22 in autoimmune lesions, we can reestablish the balance between autoreactive and regulatory T cells and stop progression of autoreactivity in the same way.

There are convincing experimental evidences supporting the suggestion that "Local elevation of CCL22 could be a treatment for organ specific autoimmune diseases".

8. Diabetes type 1

Auto-reactive T cells against insulin-producing pancreatic langerhans-islet are known in diabetes type-I. They are blocked by antibodies [29] or transferred Tregs [30]and showed good result in cessation of the progressive beta-cell damage.

Montane. J et al., 2011 prevented marine auto-immune diabetes by intra-pancreatic duct injection of double stranded adeno-associated virus encoding CCL22 [31]. This resulted in increase of CCL22 level in the pancreatic islet and recruited endogenous regulatory T cells. The result of this experiment was challenging because it resulted in prevention of β cells destruction by auto-reactive T cells, sustained the protection against auto-immune diabetes in NOD mice and delayed the relapse of diabetes. Added to that, it increased the frequency of TGF- β producing $CD4^+$ regulatory T cells around the pancreatic islet and decreased the frequency of circulating $CD8^+$, IFN- γ producing autoreactive T cells.

9. Bioinspired controlled release of CCL22

Siddharth Jhunjhunwala et al. (2012) designed a control released vehicle for CCL22 and tested their device in many mice models. The device was effectively able to delay rejection of allogenic cells transferred to the site previously injected by CCL22MP. They found excessive migration of regulatory T cells to the site of injection in response to CCL22 controlled release [32]. They finally concluded that the site-specific attraction of Tregs leading to local immunomodulation can be achieved in vivo using CCL22MP.

10. Conclusion

There are different strategies to deal with autoreactive T cells. The first one is by depletion of the activated T cells, which means any actively divided T cells. The second is by expansion of regulatory T cells and recruit them through Chemokines (like CCL22) to the specific macular area to do area specific immunosuppression. The problem with this model is in the dendritic cells stage of maturation because dendritic cells express CCL22 only up on maturation whereas the immunosuppressive therapies (like Corticosteroids and VitD3) make them in an immature stage that inhibit CCl22 expression.

We need to know more about dendritic cells chemokines and cytokines regulation in response to various sets of antigen to choose the optimum path way for expansion and recruitment of regulatory T cells so as to selectively inhibit auto reactive T cells.

Conflict of interest

We have no conflict of interest to declare.

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