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Revisiting the role of B cells in skin immune surveillance

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Whereas our understanding of the skin immune system has increased exponentially in recent years, the role of B cells in cutaneous immunity remains poorly defined. Recent studies have revealed the presence of B cells within lymphocytic infiltrates in chronic inflammatory skin diseases and cutaneous malignancies including melanoma, and have examined their functional significance in these settings. We review these findings and discuss them in the context of the current understanding of the role of B cells in normal skin physiology, as well as in both animal and human models of skin pathology. We integrate these findings into a model of cutaneous immunity wherein crosstalk between B cells and other skin-resident immune cells plays a central role in skin immune homeostasis.

Emerging roles for B lymphocytes in the cutaneous immune system

The skin is one of the largest organs in the human body and fulfills a host of functions, including acting as a protective barrier between the internal and external environments. Constant exposure to a wide array of potentially harmful insults has also necessitated the evolution of a complex and well-coordinated innate and adaptive immune network in the skin, which ensures that adequate immune responses are mounted in response to antigenic challenge while maintaining overall homeostasis [1]. Originally termed skin-associated lymphoid tissue (SALT) and subsequently renamed the 'skin immune system' (SIS), this network comprises of specialized skin-resident immune cells, as well as immunocompetent skin-trophic lymphocytes constantly recirculating between the skin, skin-draining lymph nodes, and the peripheral circulation [2,3].

Anatomically the skin is composed of two layers. The epidermis is made up of specialized epithelial cells known as keratinocytes, specialized dendritic cells (DCs) known as Langerhans cells, and $CD8^+$ cytotoxic T cells. The dermis is home to a diverse array of specialized immune cells including antigen-presenting dermal DCs, T cells, B cells, and natural killer (NK) cells, as well as mast cells, monocytes, and macrophages [2,4,5]. The contribution of different immune cell components to cutaneous immune responses has been thoroughly investigated in recent years [5–9], and these efforts have led to significant advances in the treatment of inflammatory skin conditions and skin tumors, including the use of immunobiological agents developed as a result of an improved understanding of the cutaneous immune system. Of particular note, recent developments in the treatment of metastatic melanoma aimed at stimulating the antitumor immune response have offered improved survival for patients where therapeutic options were previously very limited [10].

B lymphocytes form the humoral arm of the immune system and are responsible for the production of antibodies. They also have antibody-independent roles, acting as antigen presenting cells [11,12] and producing cytokines with potent effects on both localized and systemic immunity [13–17]. Emerging evidence indicates that B cells play important roles in skin homeostasis and disease, with both proinflammatory and immunoregulatory actions. Recent findings have revealed crucial roles for B cells within the cutaneous tumor microenvironment in melanoma [18]. Furthermore, accumulating evidence from both animal and human models of skin inflammation supports the notion that B lymphocytes are an integral part of the cutaneous immune system. We review recent findings that have provided important insights into this under-investigated component of the cutaneous immune system. We outline emerging concepts relating to the involvement of B cells in the skin immune system, and propose a model that incorporates these concepts into the current understanding of the mechanisms that regulate lymphocyte migration into the skin both in health and in disease.

Mechanisms guiding B cell migration to the skin

B cells emigrate from the bone marrow and recirculate via the blood to secondary lymphoid organs [19]. Following the initiation of an immune response, activated T and B lymphocytes preferentially migrate back to initial sites of antigen encounter [20–22]. For T and B lymphocytes, this preferential movement has been shown to be a function of the source of antigen-presenting cells (APCs) to which naive cells have been exposed to within the microenvironment of the secondary lymphoid tissues, or as a result of metabolites such as retinoic acid and 1,25-dihydroxyvitamin

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D3 synthesized by antigen-presenting DCs from inactive precursor vitamins [23–26].

As with T cells, the migration of B cells from the peripheral circulation into tissue sites is governed by interactions between selectins, chemokine receptors, and integrins with their cognate ligands (Box 1). The expression of these molecules on immune cells is a tightly regulated process, generally exhibiting a high degree of tissue specificity. Significant advances have been made in our understanding of the mechanisms that regulate the trafficking of T and B lymphocytes in the gut [27] and lymph nodes [28]. Much less is known about the mechanisms that govern B lymphocyte recruitment to the skin. Recently, chemokine receptors associated with skin homing in T cells have also been identified on B cells, suggesting that similar homing mechanisms may be employed by both cell types.

Cutaneous lymphocyte antigen (CLA) guides the migration of activated T cells into the skin [19,29]. CLA binds to E-selectin, which is upregulated on cutaneous vascular endothelium under inflammatory conditions [30,31] but is also constitutively expressed at low levels in normal uninflamed skin [32,33]. CLA is expressed on a population of human circulating, class-switched memory B cells [34–36]. B cells activated *in vitro* with phorbol myristate acetate (PMA) upregulated CLA expression and exhibited enhanced binding to E-selectin [34], indicating that appropriate stimulatory conditions can induce CLA expression in B cells, potentially enabling cutaneous migration. Geherin *et al.* [37] also found enriched expression of an E-selectin ligand on B cells isolated from sheep skin, as compared to circulating B cells. However, it should be noted that the group 'E-selectin ligands' incorporates different glycoproteins, of which CLA is one isoform [38,39]. Thus, other relevant E-selectin ligands may be expressed on B lymphocytes. In humans, primary percutaneous (intramuscular) immunization with *Salmonella typhi* or tetanus toxoid vaccines resulted in the generation of circulating antibody-secreting B cells that exhibited higher CLA expression than following immunization via oral or rectal routes [40], indicating that, as in T cells, CLA expression on B cells is likely to be dependent on the site of antigenic encounter.

Less is known about potential receptor–ligand pairs responsible for controlling subsequent steps of B cell homing to the skin. B cell migration to peripheral sites of inflammation is mediated by chemokines produced locally within tissues. For example, a significant number of B cells present within the synovial tissue in patients with rheumatoid arthritis have been shown to express chemokine receptors including CCR5, CCR6, and CXCR3 [41]. Because their respective ligands (CCL3, CCL4, and CCL5 for CCR5; CCL20 for CCR6; and CXCL10 and CXCL11 for CXCR3) are expressed within the joint synovium, interactions between these receptor–ligand pairs have been suggested to contribute to B cell migration into synovial tissue in patients with rheumatoid arthritis [41]. CCR6 is highly expressed on circulating B cells, and its expression has been associated with migration to Peyer's patches in the gut and to inflamed epithelia which express the CCR6 ligand CCL20 [42,43]. CCR6 is expressed on the majority of normal human skin-resident CLA^+ T cells [44], and CCL20 is constitutively expressed at low levels in normal skin endothelium [45], suggesting an important role for the CCR6–CCL20 axis in skin homeostasis. This axis was reported to facilitate the recirculation of B cells from skin-draining lymph nodes to the skin in a model of chronic skin inflammation in sheep [37]. Interestingly, this study also suggests that, unlike peripheral blood B cells where CCR6 responsiveness to CCL20 is contingent upon stimulation via the B cell receptor [43], B cells isolated from skin-draining lymph nodes appear to be constitutively responsive to CCL20, further highlighting the relevance of this axis in B cell skin homing [37].

The expression of CXCR3 and its ligands – CXCL9, CXCL10 and CXCL11 – is rapidly induced in an inflammatory environment rich in Th1 cytokines [46]. CXCR3 expression on $CD8^+$ T cells has been implicated in the pathogenesis of psoriasis vulgaris [47] and cutaneous graft-versus-host disease [48]. Similarly to CCR6, CXCR3 is also expressed on subsets of B cells in peripheral circulation, and has been shown to play a role in B cell migration to the joint synovium in the context of autoimmune inflammation in patients with rheumatoid arthritis [41] and juvenile idiopathic arthritis [49]. Whether CXCR3 plays a role in B cell migration to the skin during homeostasis, or whether its expression is associated only with trafficking to sites of inflammation, requires further investigation.

The chemokine receptors CCR4 and CCR10 have also been implicated in the control of T cell homing to the skin;

Box 1. Control of B lymphocyte homing to tissue sites

B cell production occurs predominantly in the bone marrow, following which cells migrate to secondary lymphoid organs such as the spleen, peripheral lymph nodes (PLN), and gut-associated lymphoid tissue including Peyer's patches (PP) and mesenteric lymph nodes (MLN). In these tissues their maturation and differentiation into immunocompetent effector cells takes place, followed by their recirculation to peripheral tissue compartments such as the gut. According to the classical model of lymphocyte trafficking, migration is coordinated by the sequential interaction between unique combinations of tissue-specific adhesion molecules (selectins and integrins), together with chemokines and their concomitant receptors, resulting in eventual transmigration of cells through vascular endothelia and into tissue sites [103]. For instance, interactions between L-selectin expressed on B cells and peripheral lymph-node addressin (PNAd) expressed on high endothelial venules (HEV) results in decelerative braking (tethering) of circulating cells, as well as sustained contact (rolling) of these cells against the vascular endothelial wall, and constitutes the initial step of B cell migration to PLN [103,104]. In the case of B cell homing to PP and MLN, these interactions also involve $\alpha4\beta7$ integrin and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) [104]. Following this initial step, arrested B cells undergo firm adhesion to vascular endothelial cells mediated via interactions between activated LFA-1 integrin (leucocyte function adhesion molecule-1) and ICAM-1 (intracellular adhesion molecule-1) [105]. Firm adhesion is however preceded by signaling between chemokine–chemokine receptor pairs (CCL19/CCL21–CCR7; CXCL12/CXCL13–CXCR4) expressed on the vascular endothelium and B cells, respectively [106,107]. In PP and MLN, homing interactions, chemokine signaling, and subsequent integrin-mediated firm adhesion additionally rely on cooperation between CXCL13–CXCR5 and $\alpha4\beta7$–MAdCAM-1 [106,107]. The exact signals that control the final stage of lymphocyte trafficking, which involves the transmigration of cells across vascular endothelia, are still incompletely understood. However multiple adhesion molecule pathways including ICAM-1, vascular cell adhesion molecule (VCAM)-1, and CD47 have been described and are still being investigated [108,109].

their ligands – CCL17 and CCL27 – are expressed in the dermal vasculature and by epidermal keratinocytes, respectively [33,50]. As with CLA, CCR4 and CCR10 are also expressed on skin-homing and skin-resident T cells in humans [44,51,52]. Expression of CCR4 has been reported on subsets of human circulating [53] and tonsillar [54] B cells. Interestingly, CCR4 expression was strongly associated with co-expression of α4β7 integrin, indicative of intestinal as opposed to cutaneous homing [53]. Whether all $CCR4^+$ B cells co-express α4β7 integrin is unclear, as is the impact of the expression of these molecules on skin versus intestinal homing.

CCR10 is important for trafficking of IgA-producing B cells to mucosal epithelial sites [42]. Oral immunization of healthy human volunteers with *Salmonella typhi* but not systemic (intramuscular) immunization with tetanus toxoid has been shown to result in the generation of $CCR10^+$ antigen-specific antibody-secreting B cells that demonstrate robust migration towards CCL28 that is expressed on the gut mucosa [22]. The active metabolite of vitamin D (1,25-dihydroxyvitamin D3) can induce expression of CCR10 in B cells [55]; because vitamin D is produced locally in the skin, B cell activation within the skin (i.e., in the presence of vitamin D) may be accompanied by CCR10 expression. Further investigation will be required to specifically establish the relevance of CCR10 in B cell homing to the skin.

B cells in normal skin

Recent years have seen much growth in the understanding of the functions of skin-resident immune cells. The number of T lymphocytes present in normal human skin has been estimated to be $\sim 2.0 \times 10^{10}$, almost twice the number of cells estimated to be in peripheral circulation [44,56]. Experiments in which peri-lesional skin from psoriatic patients was transplanted onto RAG2-deficient mice also lacking type I and II interferon receptors highlighted the presence of antigen-educated memory T cells in the skin, which were able to initiate overt psoriatic skin lesions in recipient animals [57]. These results pointed to the existence of a skin-resident memory T cell population in healthy skin, with the ability to influence disease onset and/or outcome without necessarily relying on cell emigration from the peripheral circulation. Different subsets of this tissue-resident memory T cell population (T_{RM}) have subsequently been described in other models of skin infection [58,59].

Previously published work and our own observations (unpublished) indicate that B cells occur infrequently in normal skin under homeostatic conditions [60], and thus they have proved difficult to phenotype and quantify. Approaches used to quantify other tissue-resident populations, such as immunohistochemistry, and methods for isolating tissue-infiltrating cells involving mechanical or enzymatic tissue disaggregation and skin suction blistering [61], are not well suited for the analyses of skin B cell populations given the low-number of cells. Nihal *et al.* used a combination of PCR-based analysis for immunoglobulin heavy chain rearrangements and immunoperoxidase staining to show that, although rare, B cell infiltrates were present in normal human skin [60]. Importantly, these cells also displayed a clonally restricted pattern, indicating recognition of a restricted antigenic repertoire – most likely against skin-associated antigens – alluding to the possibility of a skin-resident memory B cell population.

Animal models of afferent lymphatic cannulation have also been employed to analyze lymphocyte subsets in the skin [62,63]. Using this approach, Geherin *et al.* demonstrated the presence of B cells in normal uninflamed skin of sheep [37]. These B cells were heterogeneous in phenotype, comprising a mixed population of innate-like cells resembling previously described B-1 cells expressing IgM^{hi} and $CD11b^{hi}$, as well as activated B cells, and to have functional capabilities as evidenced by their expression of antigen-presenting and co-stimulatory molecules including MHC class II, CD80, and CD86 [37].

In summary, there is some preliminary evidence to indicate that B cell populations are present in normal skin. However, it is currently unknown whether these cells represent a specific skin-resident population or whether they are derived from circulating populations of B cells. Factors governing the maintenance of B cell populations within the skin have yet to be investigated, as have the mechanisms governing B cell circulation through the skin. Further analysis of B lymphocyte populations present in normal skin will be necessary to understand possible contributions of B cells to skin homeostasis and immunosurveillance.

Multiple roles for B cells in cutaneous inflammatory skin disease

B cells have been shown to exhibit both proinflammatory as well as suppressive roles in the pathophysiology of inflammatory skin disorders in animal models and clinical studies in human subjects (Table 1). In humans, dermal B cell infiltrates have been observed in chronic inflammatory skin conditions including cutaneous leishmaniasis, diffuse cutaneous sclerosis, and atopic dermatitis (AD) [64–66]. B cells appear to contribute to cutaneous inflammation via interactions with both innate immune cells and T cells, as discussed below.

Studies in animal models of delayed type hypersensitivity have provided evidence in support of a role for B cells in the initiation, propagation, and suppression of cutaneous inflammation. In studies performed using μMT and X-linked immune defect (XID) mice, which are pan-B cell and B-1 B cell deficient respectively, the innate-like B-1 subset of B cells was shown to contribute to the initiation of delayed type/contact hypersensitivity responses via a rapid response pathway involving the production of antigen-specific IgM antibodies. This occurred as early as 1 day post-immunization with protein antigens keyhole limpet hemocyanin or ovalbumin, and was followed by immune complex formation and subsequent activation of the complement cascade, leading to T cell recruitment at cutaneous sites of secondary antigen challenge [67,68]. Whether these mechanisms contribute to allergic reactions in humans remains to be determined because there is currently a lack of consensus as to the human equivalent of murine B-1 B cells [69–71].

B cells are also likely to play a role in chronic inflammatory conditions such as AD. CD19-deficient ($CD19^{-/-}$)

Table 1. Phenotype of B cell subsets with proposed roles in skin immunity[a]

Phenotype	Proposed effector function/clinical significance	Pathology
Animal studies		
B-1-like B cells – $CD11b^{hi}$ IgM^{hi}	Skin surveillance	Unperturbed sheep skin [37]
B-1-like B cells – $CD11b^{hi}$ IgM^{hi}; $CD80^{+}/86^{+}$, $CD1^{+}$, $CCR6^{+}$	Antigen presentation, local antibody production	CFA[b]-induced chronic sheep granuloma [37]
$CD19^{+}$ B cells (?B10 subset)	Suppression of CHS responses via production of IL-10	DNFB-induced murine CHS [110]
$CD19^{hi}CD1d^{hi}CD5^{+}$ regulatory B cell subset	Negative regulation of T cell mediated inflammatory responses via IL-10 production	Oxazolone-induced murine CHS [86]
$CD19^{+}CD1d^{hi}CD5^{+}$ regulatory B cell subset (B10 cells)	Suppression of skin inflammation via production of IL-10	Imiquimod-induced murine Psoriasis [85]
$CD19^{+}CD21^{hi}$ (transitional zone B cells)	Suppression of anti-tumor immunity via production of IL-10	DMBA/TPA-induced murine squamous cell carcinoma [94]
$CD19^{+}CD22^{+}CD5^{+}$ B-1 B cells	Initiation of contact sensitivity responses via complement activation stimulated by rapidly produced IgM–Ag complexes	Oxazolone or picryl chloride-induced murine CHS [67] Keyhole limpet hemocyanin or ovalbumin-induced murine DTH [68]
$CD19^{+}$ B cells	Enhancement of antigen-specific $CD4^{+}$ T cell activation and expansion; Enhancement of Th2 and Th17 T cell responses	Ovalbumin-induced murine AD [72]
$CD19^{+}CD20^{+}$ B cells	Immunoglobulin-mediated tumor development via activation of Fcγ receptors on resident and recruited myeloid cells	Squamous cell carcinoma [93]
$CD19^{+}CD20^{+}$ B cells	Pre-treatment of tumor-bearing mice with Rituximab improves response to platinum- and Taxol-based chemotherapy	Squamous cell carcinoma [90]
Human studies		
$CD20^{+}$ B cells	Clinical improvement in skin lesions following depletion of B cells in lesional skin using Rituximab Clinical improvement in skin lesions following use of Rituximab Induction of psoriasis following B cell depletion with Rituximab	Severe atopic eczema [66] Severe atopic dermatitis [73] Psoriasis [88,89]
$CD20^{+}$ B cells	Correlation between low number of $CD20^{+}$ tumor-infiltrating B cells and disease progression	Primary cutaneous melanoma [91]
$CD22^{+}$ B cells	Production of IgG4 antibodies in tumor lesions which impair antitumor immunity	Cutaneous melanoma [18]

[a]See associated references and discussion in the main text.

[b]Abbreviations: AD, atopic dermatitis; CFA, complete Freund's adjuvant; CHS, contact hypersensitivity; DTH, delayed type hypersensitivity; DMBA/TPA, 7,12-dimethylbenz(α)anthracene/terephthalic acid; DNFB, 2,4 dinitro-1-fluorobenezene.

mice sensitized with ovalbumin via the epicutaneous route were found to display a less-severe histological AD phenotype (assessed by skin thickening) compared to wild type (WT) mice. In addition, these mice exhibited reduced proliferation of antigen-specific $CD4^{+}$ T cells as well as reduced secretion of Th2 and Th17 cytokines [72]. Furthermore, adoptive transfer of WT B cells into CD19-deficient mice resulted in severe AD, pointing to a pathogenic role for $CD19^{+}$ B cells in the development of AD in this model. However, although the $CD19^{-/-}$ mice were not depleted of B cell subsets, they have reduced overall B cell numbers, with reduced B cell proliferation in response to mitogens. Further work will therefore be necessary to establish the precise role for CD19 in the context of atopic dermatitis.

In humans, treatment with Rituximab, a chimeric monoclonal antibody that targets the B cell surface antigen CD20 and depletes $CD20^{+}$ B cells, has been reported to improve AD lesions as assessed by cutaneous symptoms as well as histopathological parameters including acanthosis and hyperkeratosis [66,73]. Thus, whereas AD is considered to be a T cell driven disease, these findings suggest a role for B cells in exacerbating AD pathology. It is currently unclear whether this role is related to antibody production by B cells, through an impact of B cells on T cell function, or both.

Autoimmune bullous disorders are dermatological diseases characterized by the presence of circulating autoantibodies directed against structural proteins of the epidermis or dermo-epidermal junction [74]. These conditions are commonly treated with systemic glucocorticoids in combination with either immunosuppressants such as Azathioprine or Mycophenolate mofetil or immunomodulatory agents including Dapsone and intravenous immunoglobulins. However, because not all patients respond to these treatments, B cell depletion therapy using Rituximab (with a view to targeting autoreactive B cells) has been evaluated in a limited number of patients. Treatment of immunobullous disorders including pemphigus and bullous pemphigoid with Rituximab resulted in improvement of skin lesions, and has been reported to be associated with a reduction in titers of circulating autoantibodies in some patients, and this was attributed to the systemic depletion of mature $CD20^{+}$ B cells [75–81].

B cells have also been found to have a suppressive role in particular settings of skin inflammation. Early studies in guinea pigs intradermally injected with hen egg albumin conjugated to the hapten para-aminobenzoic acid demonstrated that adoptive transfer of splenic B cells from pre-sensitized donors was able to suppress delayed type hypersensitivity responses in similarly sensitized recipients, leading the authors to propose the existence of a

population of suppressor B cells [82]. Recent studies have suggested that this suppressor or regulatory B cell population, termed 'B-reg cells', has multiple roles in the modulation of systemic immune responses (reviewed in [83,84]). Whereas there is currently no consensus as to the phenotype of these cells in humans, as defined by cell surface markers and the expression of transcription factors, B-reg cells are uniformly characterized by their ability to produce the anti-inflammatory cytokine interleukin-10 (IL-10).

The ability of IL-10-producing B cells to ameliorate cutaneous inflammatory responses has been demonstrated in murine models of skin inflammation. In an imiquimod induced model of psoriasis-like inflammation, skin inflammation was found to be more severe in $CD19^{-/-}$ mice, which are also deficient in an IL-10-producing subset of $CD1d^{hi}$ $CD5^{+}$ regulatory (B10) B cells, than in WT mice. Adoptive transfer of WT B10 B cells to $CD19^{-/-}$ mice was found to ameliorate cutaneous inflammation [85] These findings were also replicated for the same animal model and subgroup of $CD1d^{hi}$ $CD5^{+}$ regulatory B cells in the context of contact hypersensitivity [86]

Although a B10 subset of B cells has been identified in humans [87], there are currently no published data available on this subset in the context of human skin, and further investigation will therefore be necessary to establish a role for this subset in human cutaneous immune responses. Interestingly, however, treatment with Rituximab was recently reported to result in the development of psoriasis in patients undergoing B cell depletion therapy for other indications including rheumatoid arthritis, systemic lupus erythematosus, or non-Hodgkin lymphoma [88,89]. In addition to the combination of environmental triggers and genetic factors that confer susceptibility to the development of psoriasis, immune cells including T cells have been shown to participate in the dysregulated immune responses that underlie this disease [6]. Because the Rituximab-treated patients in these studies had no previous family history of psoriasis, nor had they been exposed to risk factors associated with the development of this disease [88,89], these findings suggest that B cells might also play a suppressive role in the development of T cell mediated human inflammatory skin diseases.

It is presently unclear as to why B cell depletion in the context of certain forms of skin inflammation such as AD or immunobullous disease is beneficial in reducing inflammation, whereas in other settings it conversely induces an inflammatory response such as in psoriasis. $CD20^{+}$ B cells encompass both effector and regulatory B cell subtypes, and therefore Rituximab could in theory deplete both pro- and anti-inflammatory subsets of B cells. The apparently conflicting observations in human skin inflammation may be as a result of different contributions of effector and suppressor B cells to the pathogenesis of specific diseases. An improved understanding of the phenotype of human IL-10-producing cells in the setting of different forms of skin inflammation and the effects of immune targeted therapies on different B cell subsets will be necessary to explain these observations further.

Thus, the available evidence supports a role for B cells in inflammatory skin disease, although the mechanisms involved, which appear to be distinct in different disease settings, remain undefined.

Pro- and anti-inflammatory roles for B cells in cutaneous tumors

B cells are present in tumor-infiltrating lymphocyte (TIL) populations in several cutaneous malignancies including squamous cell carcinomas (SCCs) and melanomas [18,90,91]. Evidence from patient samples and experimental models of skin malignancies suggest that infiltrating and circulating B cells may play multiple roles in different contexts, including participation in the adaptive response to tumor growth, and also in chronic inflammation that may promote tumor progression.

In SCC models, B cells have been demonstrated as being crucial to the initiation of chronic inflammation in murine premalignant skin lesions [92]. A *HPV16*/*Rag1*$^{-/-}$ mouse model of *de novo* epithelial carcinogenesis, based on expression of human papillomavirus type 16 (HPV16) under the control of the human keratin 14 promoter, showed lack of chronic inflammation and reduced incidence of SCC and associated inflammatory infiltrates when the mice were bred onto a RAG1-deficient background which featured absence of mature T and B cells. Importantly, adoptive transfer of B cells to *HPV16*/*Rag1*$^{-/-}$ mice, or of immunoglobulin-containing serum from *HPV16* mice, was sufficient to restore inflammatory cell infiltration into premalignant tissues and for the transition into full malignancy [92]. Subsequent studies revealed that development of SCC in these animals was mediated via the activation of tumor-promoting myeloid-derived suppressor cells (MDSC) by cutaneous immunoglobulin deposits, and that B cell depletion therapy resulted in an improved tumor response to chemotherapy [90,93]. Interestingly, IL-10 production by regulatory B cells has also been suggested to impair antitumor immunity in a dimethybenz(α)anthracene/terephthalic acid (DMBA/TPA) mouse model of skin squamous carcinogenesis [94].

The mechanisms governing the role of B cells in antitumor immunity to melanoma tumors are not fully elucidated. In mouse models of melanoma, B cells may promote tumor growth by supporting angiogenesis and lymphangiogenesis [95,96]. Emerging evidence also points to systemic as well as tumor-resident B cell responses in human cutaneous melanomas; B cell infiltrates are detected in melanoma lesions, and lymphoid-like structures rich in B cells are observed in some primary and metastatic melanomas [97–99]. Ladanyi *et al*. have reported correlations between the densities of tumor-infiltrating B cells and more favorable clinical outcomes in patients with cutaneous melanomas [91]. These suggest that there are active adaptive B cell immune responses in the malignant melanoma skin microenvironment. In the circulation, higher frequencies of melanoma tumor-reactive IgG antibodies derived from mature memory B cells have been detected in patients with melanoma compared to those from healthy volunteers [100]. Despite mounting evidence of humoral immune surveillance in melanoma, however, the balance is tipped in favor of tumor growth, possibly as a consequence of immunosuppressive mechanisms that may modulate B cell functions. We previously demonstrated the presence of

mature ($CD22^+$) antibody-producing B cells within human primary and metastatic cutaneous melanoma lesions. We reported that melanomas support Th2-biased inflammatory conditions, featuring IL-10 and VEGF, that favor production of IgG4 subclass antibodies by B cells [18]. We showed that IgG4 antibodies have limited effector functions against tumor cells, and can also block otherwise tumoricidal IgG1 antibodies from activating immune effector cells against melanoma tumors, pointing to a previously unidentified potential mechanism of tumor immune escape. Consistent with altered or dysregulated humoral responses in melanoma, elevated serum IgG4 levels were also associated with worse clinical outcomes in patients. Furthermore, we recently identified a small subset of

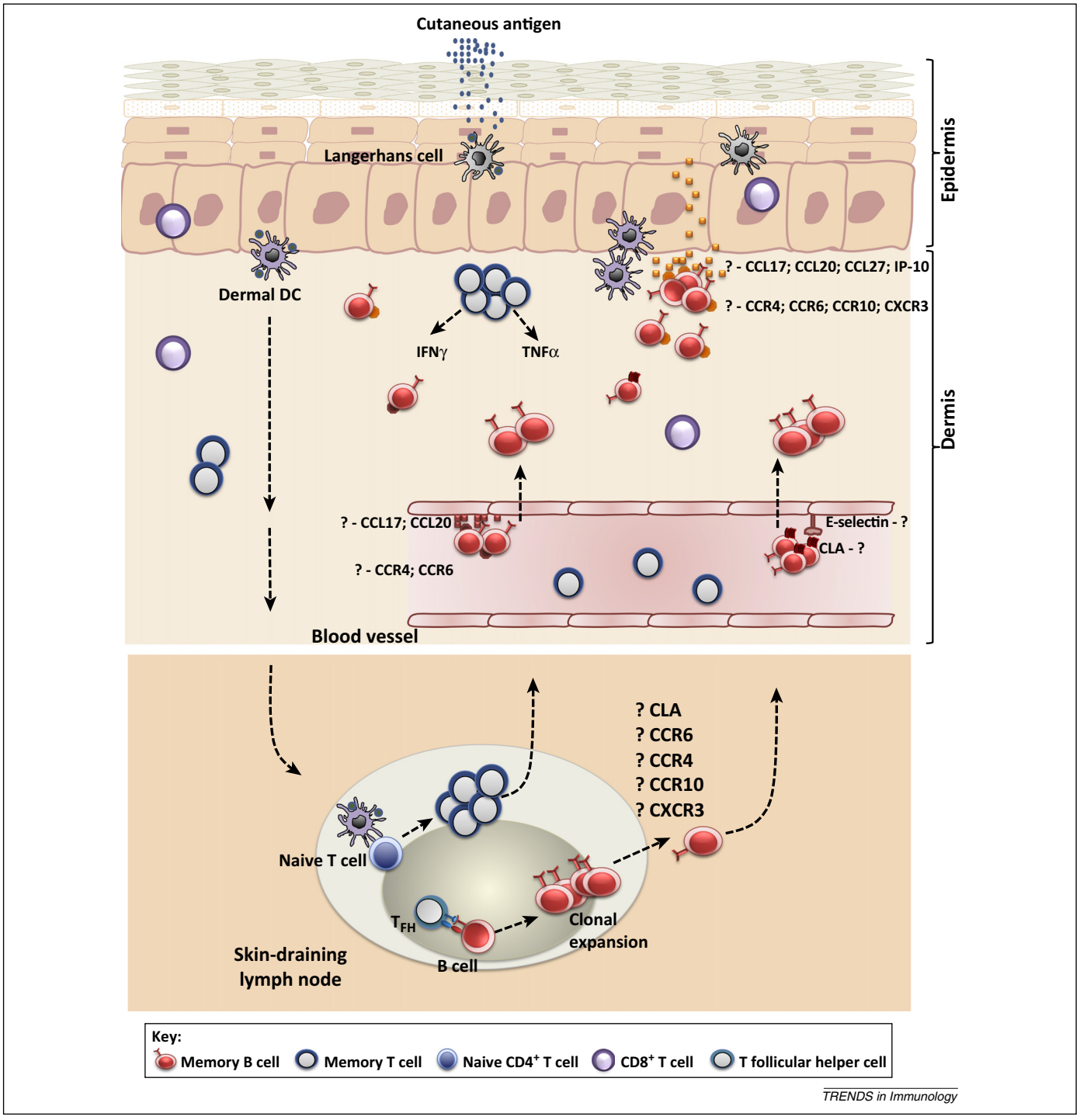

Figure 1. Model of B cell migration to cutaneous sites. A diverse array of immune cells are present within the skin including Langerhans cells, dermal dendritic cells (DCs), B cells, and T cell subsets. Naïve T cells are primed via presentation of cutaneous antigens by dermal DCs within skin-draining lymph nodes. Activated T cells subsequently migrate back to the skin and can produce proinflammatory cytokines (e.g., IFNγ and TNFα) as part of a cutaneous immune response. Similarly, we postulate that possible interactions between cutaneous antigen-specific B cells and appropriately primed follicular helper T cells (T_{FH}) within skin-draining lymph nodes may result in generation of a memory B cell population which expresses/upregulates skin-homing chemokine receptors (such as CLA, CCR4, CCR6, CCR10, and CXCR3) and subsequently traffics into the skin. At the same time, receptor interaction with cognate ligands including E-selectin and CCL17 (from dermal blood vessels) or CCL27 (from epidermal keratinocytes), which are constitutively expressed or induced within the skin in response to proinflammatory signals, may additionally serve to direct the trafficking of activated B cells through cutaneous vascular endothelia and into the dermis.

IL-10-producing B lymphocytes within human cutaneous melanoma metastases, indicative of a population of regulatory B lymphocytes in melanoma; the precise phenotype and specific functions of this population have yet to be fully delineated [101].

Overall, these findings paint a complex picture of tumor crosstalk with B cells and describe tumor-associated inflammatory conditions that may modulate humoral responses possibly impairing tumor clearance, and suggest the functional relevance of B lymphocytes to the pathogenesis, progression, and prognosis of cutaneous tumors.

Concluding remarks

Growing evidence supports a role for B cells in cutaneous immunity both in homeostasis and during inflammation associated with infection, inflammatory disease, and skin tumors. The existing paradigm of the skin immune system involves the concerted actions of specialized cellular effectors, including dendritic cell and T cell subsets, in maintaining a fine balance between immune protection and the development of autoimmunity. We would like to propose a model wherein B cells constitute an integral part of this cellular network, fulfilling both antibody-dependent and

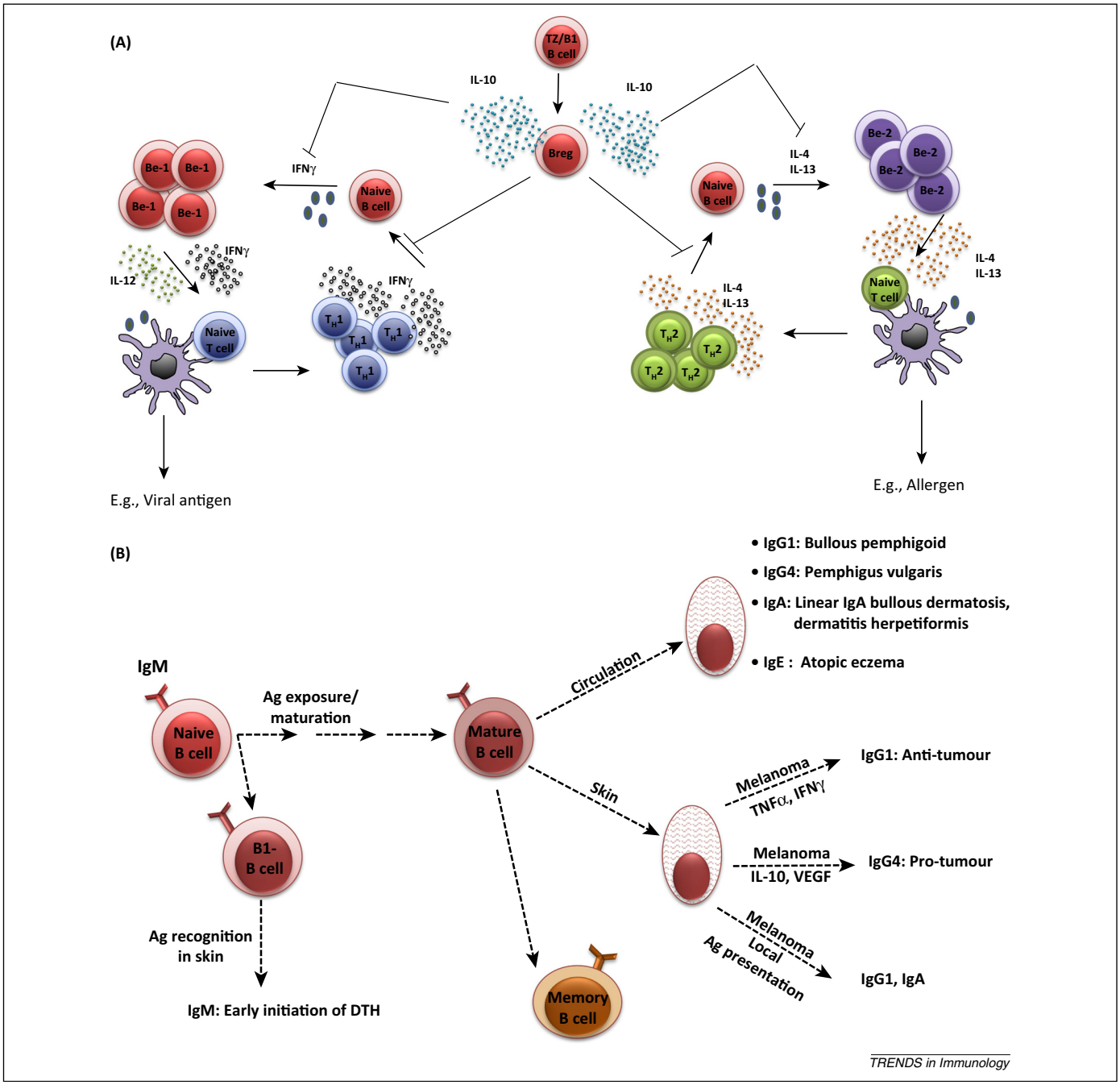

Figure 2. B cell roles in skin immunity. **(A)** B cells may modulate skin immune responses through their production of pro- and anti-inflammatory cytokines. Naïve B cells primed in the presence of specific antigens (Ag) and T_H1 or T_H2 cytokines are able to adopt the same 'effector phenotype' as T cells. Be-1 (B cells primed in a T_H1 environment) and Be-2 (B cells primed in a T_H2 environment) cells act as a source of positive feedback by producing cytokines that maintain ongoing inflammation. Splenic transitional zone (TZ) and B1-B cells are thought to differentiate into regulatory B cells (Bregs). Adapted from [13]. **(B)** Modulation of cutaneous pathology by B cells can take place in the context of systemic (e.g., immunobullous disease) or local (e.g., tumor microenvironment) antibody production. Early production of low-affinity IgM antibodies also occurs in delayed type hypersensitivity (DTH) responses.

independent roles in the maintenance of skin immunity as well as in its defense against tumors and invading pathogens (Figure 1).

B lymphocytes not only express skin-homing markers, indicative of an ability to home to sites of cutaneous inflammation, but have also been identified in both normal skin and in cutaneous pathology. In addition to involvement in the early stages of skin immune responses via the production of non-specific antibodies [67,68], mature B cells also contribute to the skin-specific immune response by producing antibodies both locally and within the circulation [18,100]. In addition, they may mature to become cytokine-secreting effector B cells which are able to modulate skin immune responses via the production of pro- or anti-inflammatory cytokines, dependent on the milieu of the pre-existing microenvironment in the skin (Figure 2).

The presentation of cutaneous antigens to naïve T cells is known to occur within skin-draining lymph nodes, with subsequent re-exposure to memory T cells occurring within the skin itself [102]. Naïve B cells are also known to transit through secondary lymph nodes, including those draining the skin [37], where it seems likely that they will encounter cutaneous antigens. However, it remains unknown whether this eventually leads to the generation of skin-specific B cells. It is possible that antigen presentation to B cells may occur locally in the skin, at least within the context of cutaneous tumors [98]. The presence of tertiary lymphoid structures has recently been described within the context of the tumor microenvironment in primary melanomas and cutaneous metastases located within the dermis [97,98]. The association of B cell clusters with T cells, dendritic cells, and high endothelial venules within these lymphoid structures, as well as evidence of ongoing local antigen-driven B cell responses, point to the possibility of localized antigen presentation [98].

Many questions remain unanswered in this emerging area of B cell immunology. What signals determine the migration and maintenance of this population in the skin? What subsets are important in the maintenance of cutaneous immunity? Are there B cell molecular signatures which determine their effector functions in the skin, and can these signatures be targeted for diagnostic, therapeutic, or prognostic purposes? (Box 2). The skin presents us with an ideal system for the study of human immune responses both at the local and systemic levels [4]. As has been done with T cells, this resource can be utilized to provide a fuller understanding of the origins and functions of B cells in both physiology and disease.

Box 2. Important areas for future research

- Establishment of experimental human and animal model systems which allow extensive phenotyping and assessment of the functional capacity of B cell subsets in skin in the context of both immune surveillance and disease.
- Identification of definitive adhesion molecule and chemokine receptor combinations involved in B cell trafficking to skin.
- Use of lineage-tracing animal models to enhance understanding of molecular cues which determine B cell migration to skin, as well as of the crosstalk occurring between B cells and other immune cell subsets within the skin immune system
- Investigation of the antigenic specificity of B cells involved in cutaneous disease models.
- Targeting of distinct skin-relevant B cell subpopulations for therapeutic intervention in patients with cutaneous pathology.

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