Role of chemokines and chemokine receptors in regulating specific leukocyte trafficking in the immune/inflammatory response

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ABSTRACT

Antigen recognition, lymphocyte priming and effector responses in inflamed tissues depend on a coordinated and sequential series of events that take place in different anatomical compartments. The integration of these processes is favoured by the dynamic capacity of leukocytes to recirculate between the bloodstream and specific organs and to navigate inside the tissues in a programmed fashion, regulated by a complex interaction of cell adhesion molecules and soluble chemoattractants, in particular chemokines. In this review we discuss the role of chemokines and chemokine receptors in regulating leukocyte trafficking in different anatomical sites in the context of distinct functional phases of the immune/inflammatory response.

Introduction

Chemokines (CK) are low molecular weight (8-14 Kd), mostly basic, structurally related, secreted or membrane bound proteins (1). Their main activity is cell attraction and because of this property, CK are constitutively produced in organs where cell mobilization is required for the maintenance of local homeostasis. For example, haematopoiesis in bone marrow, lymphocyte education in the thymus, immune response organisation in secondary lymphoid organs as well as in embryogenesis and neurogenesis (2). On the other hand, CK can be induced or upregulated in the context of inflammation, where they behave as pro inflammatory mediators, promoting leukocyte migration and favouring the recruitment, antigen encounter and cooperation of different leukocyte subsets in peripheral tissues (2).

Up to date, more than 40 CK have been characterised (1). They share a tertiary structure stabilized by four cysteine residues that form disulphide bonds. CK have been classified in different subfamilies (Table I) on the basis of the arrangement of these cysteine residues, which can be adjacent to each other (CC chemokines), separated by one (CXC chemokines) or three amino acids (CXXXC chemokines) (1). A protein with two instead of four conserved cysteines (lymphotactin) has also been described (C chemokine) (3). The effect of CK on target cells is mediated by seven transmembrane domain receptors (CKR) coupled to trimeric G proteins. This structure characterizes the G-protein-coupled receptor superfamily (GPCR), which includes receptors for many other signalling molecules such as hormones and neurotransmitters. The binding of a CK on its cognate CKR can induce a wide spectrum of effects. These include activation and clustering of cell adhesion molecules (integrins), which facilitate the firm adhesion of leukocytes to the endothelial surface, or the process of actin polymerization and breakdown, which favours cytoskeletal rearrangements and cell mobilization (4). Due to these effects of CK, leukocytes can recirculate from the bloodstream to specific organs and move inside the tissues in a programmed fashion, regulated by a spatial and temporal succession of different CK gradients (5, 6) (Fig. 1). Of note, the activity of CK is not restricted to cell mobilization as they can directly participate to cell activation inducing the release of the content of cytoplasmic storage granules or upregulating the expression of soluble and membrane bound molecules (7, 8). In this review we will describe first the role of chemokines and chemokine receptors in regulating homeostatic trafficking to secondary lymphoid organs, followed by their specific action in the genesis of the immune response. We will then review their role in regulating the trafficking of effector cells and in the process of lymphoid neogenesis that is observed at chronic inflammatory sites.

Role of chemokines and chemokine receptors in regulating naïve lymphocyte homeostatic trafficking to secondary lymphoid organs

Under homeostatic conditions, naïve T and B lymphocytes recirculate from the bloodstream to secondary lymphoid organs where they can interact with one another and with different subsets of antigen presenting cells. If no specific antigenic stimuli are present, they exit secondary lymphoid organs through the efferent lymphatics and, via the thoracic duct, regain the systemic circulation perpetuating their route (9, 10). Circulating naïve lymphocytes enter peripheral lymph nodes mainly through specialised vessels with the characteristic high endothelial venule morphology (HEV). Although most of the circulating leukocytes can make contact with HEV, under homeostatic conditions only few of them (mainly naïve lymphocytes) are allowed to migrate inside lymphoid tissues. This selectivity is due to the interaction between specific CKR expressed by lymphoid homing cells and CK constitutively expressed in secondary lymphoid organs (11). The best candidate CK in favouring naïve lymphocyte homing to secondary lymphoid tissues are SLC/CCL21 and ELC/CCL19, which are constitutively produced by stromal cells, dendritic cells and endothelial cells in the T cell area of secondary lymphoid organs (12). They function as ligands for the CKR CCR7 expressed, in humans, by all naïve lymphocytes but absent on the surface of granulocytes, monocytes and a proportion of effector lymphocytes (13). The importance of SLC and ELC in triggering CCR7 positive lymphocyte homing to secondary lymphoid tissues is supported *in vivo* by the relative absence of naive T cells in the lymphoid tissues of plt/plt mice, a spontaneous mutant strain deficient in SLC and ELC genes (14, 15) and by the similar phenotype produced by a targeted mutation in CCR7 (16). The critical role of SLC and ELC in promoting lymphocyte trafficking in lymph nodes have been further confirmed by recent experiments which could demonstrate by intravital microscopy the inhibitory effect of CCR7 desensitization in naïve T cell adhesion to HEV endothelial cells in normal mice and the efficacy of SLC and ELC to restore naïve T cell adhesion and trafficking when singularly injected in plt/plt mice (17, 18). It is interesting to note how B and T cells exhibit differences in secondary lymphoid organ homing properties. Even if both naïve T and B cells respond in vitro to SLC and ELC in chemotaxis assays (19), B cells are present in relatively normal quantities in plt/plt mice (15). This aspect has been recently addressed by Cyster and colleagues (20) who provided evidence of the efficacy of SDF-1/CXCL12 (CXCR4 ligand, another CK constitutively expressed in the T cell areas of secondary lymphoid organs) in providing triggering signals for B cells entry to murine lymph nodes, having the potentiality to act as a functional substitute for SLC/ELC in a fully redundant fashion. The same authors could also demonstrate that SDF-1-CXCR4 was also capable of promoting T cell homing to lymph nodes, although SLC/ELC-CCR7 played a dominant role. Recently, our group have shown how SDF-1 injected in human lymph nodes transplanted into SCID mice is able to

Table I. Human CXC, C, CC and CX3C chemokines and relative receptors.

Chemokines	Systematic name	Chemokine receptors
GRO α	CXCL1	CXCR2
GRO β	CXCL2	CXCR2
GRO γ	CXCL3	CXCR2
ENA-78	CXCL5	CXCR2
GCP-2	CXCL6	CXCR2, CXCR1
NAP-2	CXCL7	CXCR2
IL-8	CXCL8	CXCR2, CXCR1
Mig	CXCL9	CXCR3
IP-10	CXCL10	CXCR3
I-TAC	CXCL11	CXCR3
SDF-1	CXCL12	CXCR4
BCA-1	CXCL13	CXCR5
Lymphotactin	XCL1	XCR1
MCP-1	CCL2	CCR2
MCP-2	CCL8	CCR3
MCP-3	CCL7	CCR1, CCR2, CCR3
MCP-4	CCL13	CCR2, CCR3
MIP-1α	CCL3	CCR1, CCR5
MIP-1 β	CCL4	CCR5
RANTES	CCL5	CCR1, CCR3, CCR5
EOTAXIN	CCL11	CCR3
EOTAXIN-2	CCL24	CCR3
EOTAXIN-3	CCL26	CCR3
LARC	CCL20	CCR6
TECK	CCL25	CCR9
CTACK	CCL27	CCR10
TARC	CCL17	CCR4
MDC	CCL22	CCR4
DC-CK1	CCL18	?
ELC	CCL19	CCR7
SLC	CCL21	CCR7
Fractalkine	CX3CL1	CX3CR1

Adapted from Zlotnik A and Yoshie O: Immunity 2000; 12: 121-7

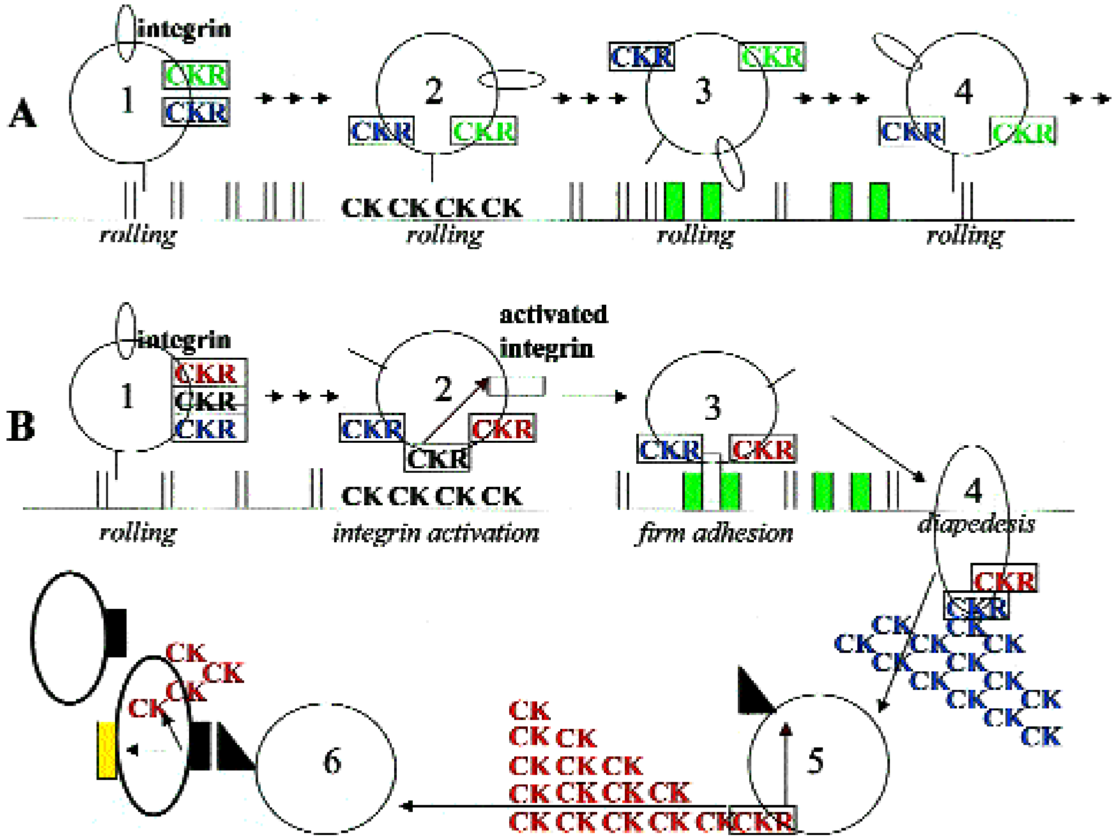

Fig. 1. Schematic representation of the role of CK and CKR in leukocyte mobilization and activation. Lines A and B show the multistep cascade involved in leukocyte-endothelial interactions, which regulate the recruitment of leukocytes from the bloodstream into the tissues.

In the first step (A1 and B1) leukocytes establish transient contacts with the endothelial surface (tethering and rolling) through the interaction of selectin molecules (thin vertical lines) that bind to oligosaccharide ligands (double thin vertical lines). These molecules can be expressed in a reciprocal fashion both by leukocytes and endothelial cells. This step does not require cell activation and is independent of the CKR pattern expressed by leukocytes (different colours represent different CKR).

In the second step (A2 and B2), only the leukocytes presenting CKR specific for the CK displayed by the endothelium can progress further in the cascade (integrin activation) with clustering and affinity increase of integrin molecules (e.g. LFA-1, $\alpha4\beta1$). This favours the arrest of the leukocytes on the endothelial surface (firm adhesion), by means of interaction between activated integrins and their endothelial ligands (e.g. ICAM-1,VCAM-1) (double green squares) (B3). The leukocytes whose surface integrins have not been activated do not arrest and carry on rolling on the endothelium (A3 and A4), while the formation of strong bonds enable leukocytes to begin the process of vascular transmigration (diapedesis) (B4). Once in the tissue, leukocytes move and position themselves in specific areas, driven by sequential chemotactic gradients, which can involve different CKR (multistep navigation model) (B5 and B6). CK also participate in the activation of leukocytes, favouring the release of soluble molecules or the expression of membrane bound molecules (purple triangle) (B5) that leads to downstream effects on resident cells (B6).

favour human peripheral blood lymphocytes migration to lymphoid tissue, suggesting a similar potential role for SDF-1 in triggering lymphocyte homing to lymphoid tissue *in vivo* also in humans (21).

Role of chemokines and chemokine receptors in regulating specific leukocyte trafficking within secondary lymphoid organs: Genesis of the immune response

Several studies performed in mouse models have highlighted the central role of CK in maintaining the organizational structure of secondary lymphoid organs. CK mainly act by directing specific leukocyte populations to discrete compartments and by favouring interactions between distinct cell populations. This provides a coordinated interactive program, functional in the genesis of the specific immune response. Two main steps can be identified: the encounter-cooperation between locally migrated naïve lymphocytes and different subset of antigen presenting cells, and the encounter-cooperation between locally activated T and B cells.

Due to the combined action and selective expression in specific areas of a group of "lymphoid homing CK" (T cell area express SLC and ELC, while B cell areas express BLC/BCA-1/ CXCL13) (Fig. 2, A and B), T and B cells migrate in distinct compartments of secondary lymphoid organs where they can interact with the appropriate type of antigen presenting cells (12). In particular BLC/BCA-1, which functions as a ligand for the CKR CXCR5, expressed in humans by naïve B cells but not naïve T cells (13), promotes the selective recruitment of B cells in B cell follicles where antigen is displayed by follicular dendritic cells (FDC). On the contrary, SLC and ELC, which as above described favour the migration of naïve lymphocytes from the bloodstream to the lymph node, promote the localization of naïve T cells in the surrounding T cell area and their interaction with mature, antigen loaded, dendritic cells (Fig. 2 C). Although the migratory habits of FDC (or their precursors) are currently unknown, several *in vitro* and *in vivo* studies have suggested that dendritic cell migration to the T cell area is a CK driven process, implying a time-ordered expression of different CKR as well as CK production. *In vitro* differentiated human immature dendritic cells are characterized by the expression of multiple CKR (CXCR1, CCR1, CCR2, CCR5 and CCR6, see Table I for ligands) specific for CK produced under inflammatory conditions (13, 22, 23). This CKR pattern can favour their recruitment from the bloodstream to inflamed tissues where the respective CK ligands are abundantly produced. Once activated by inflammatory stimuli, such as TNF-α, IL-1, bacterial or viral products they undergo a process of maturation. This involves an enhanced expression of costimulatory molecules (CD80, CD86) and an increased antigen presentation capacity. In addition, mature dendritic cells switch their CKR expression pattern and CK responsiveness. They downregulate the above described CKR for inflammatory CK and upregulate receptors responsive to CK produced within secondary lymphoid organs, such as CCR7 (22, 23). Thus, upon maturation, dendritic cells can acquire a CKR driven tropism for secondary lymphoid organs and migrate through afferent lymphatic vessels (24, 25) (Fig. 2C). The proposed model for naïve T cell and dendritic cell migration provides a clear example of flexibility and coordination of the CK-CKR system. Immature dendritic cells are first recruited by the action of inflammatory CK to peripheral tissues where they can capture antigens. Subsequently, the inflammatory environment favours their maturation that is associated to an enhanced antigen presentation capacity and to the acquisition of CKR for CK produced in secondary lymphoid organs. Due to this process they can migrate to the lymphoid tissue and exert their APC function for naïve cells which have been locally recruited by the same CK (SLC and ELC) (Fig. 2 C).

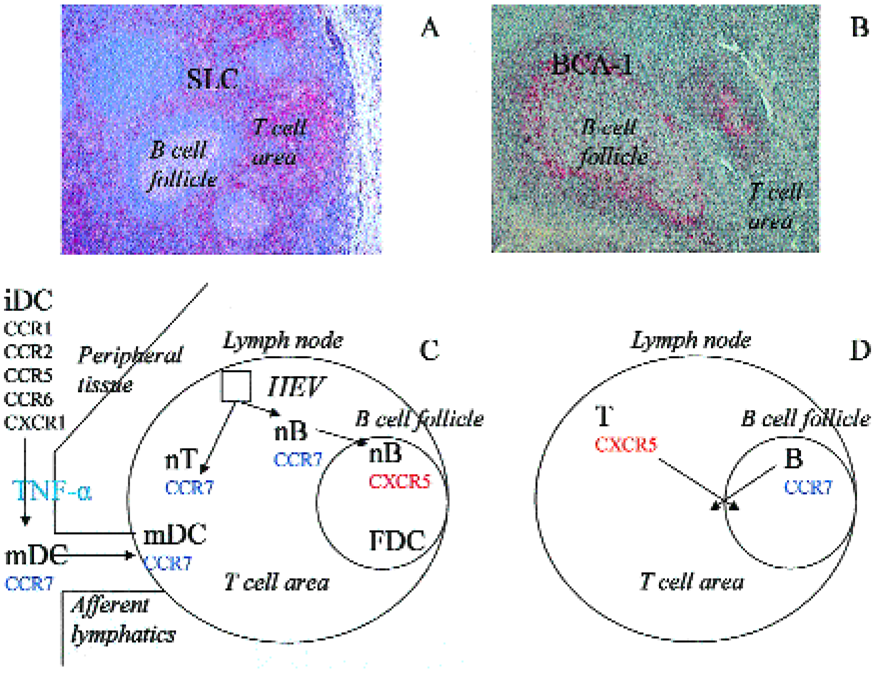

Fig. 2. (**A**) Immunostaining for SLC (in red) in human tonsil. SLC protein is localized in the T cell rich areas surrounding B cell follicles (original magnification 100X). (**B**) Immonostaining for BCA-1 (in red) in human tonsil. BCA-1 protein is localized in the B cell rich follicles but not in the T cell areas (original magnification 200x). (**C**) Schematic role of SLC and BCA-1 in dendritic cell,T cell and B cell migration and compartmentalization within specific areas of secondary lymphoid organs. SLC (and ELC) mediate the localization of CCR7 positive T lymphocytes and mature dendritic cells in the T cell rich areas, while BCA-1 favour the recruitment of CXCR5 positive B lymphocytes in follicles where they can interact with follicular dendritic cells. (**D**) Role of BCA-1 and SLC in T-B cell encounter and cooperation within secondary lymphoid organs. A switch in CKR expression by activated T and B cells favours their concomitant recruitment at the follicular-T cell rich border area. (See text for further details). iD= immature dendritic cells; mDC= mature dendritic cells; nT, naïve T cells; nB, naïve B cells; HEV, high endothelial venule.

Once activated by the appropriate type of antigen presenting cells in different compartments, T and B cells need to cooperate for an efficient generation of the T dependent specific humoral response. Again, this process can be explained by a synchronous switch in CKR usage. Activated T cells, stimulated by dendritic cells in the T cell areas upregulate CXCR5 (BLC/BCA-1 receptor) and downregulate CCR7 function (26). They become sensitive to BLC/BCA-1, expressed within B cell follicles, moving towards the B cells areas (follicular homing T cells). On the opposite side, B cells, once activated by antigens, upregulate CCR7 expression, moving towards the T cell area (27). The reciprocal exchange of CKR usage can lead to the encounter of T and B cells at the follicular border area (28) ensuring a coordinated process of cell interactions, important for the physiological genesis of the immune response (Fig. 2 D).

Role of chemokines and chemokine receptors in regulating specific leukocyte trafficking to inflammatory sites: Effector response

During an immune/inflammatory reaction, effector cells and antigen presenting cells (effector memory T lymphocytes, granulocytes, monocytes and dendritic cells) need to be recruited to the site of tissue injury. This is possible due to the capacity of several inflammatory mediators (such as TNF-α) to favour the upregulation of a series of inducible CK that promote endothelial adhesion and intratissue localization of inflammatory cells (29-31). For example, human monocytes and granulocytes, similarly to what described for immature dendritic cells, constitutively express receptors for many different inflammatory CK (13). Because of this they can be readily recruited from the bloodstream, forming a “ready to use pool” of circulating cells acting as a first line of defence (effector leukocytes) or initiating the antigen presentation process (antigen presenting cells). In contrast, as mentioned above, naïve T and B lymphocytes are programmed to recirculate to secondary lymphoid organs where they acquire effector proprieties (effector memory T cells and plasma cells) and peripheral tissues homing capacity only after a differentiation phase, which takes place within this specialised tissue. Once again, the modification of the migratory behaviour of effector versus naïve lymphocytes can be explained, at least in part, by a switch of CKR and adhesion molecules usage induced upon maturation. Furthermore, it is important to highlight that the process of lymphocyte differentiation, as well as their CKR usage modification, can be modulated at different parallel and sequential levels, depending on the cell subpopulation, the required typology of effector response and the target tissue specificity.

Once activated by T cells, some B lymphocytes proliferate and differentiate out of the follicle, while others give rise to the germinal centre reaction with the development of memory B cells and plasma cells producing high affinity, isotype switched Ig. Plasma cell trafficking seems to be regulated by a CKR specific modulation. Recent experiments in mice have shown how naïve B cells (which express functional CKR for the lymphoid homing CK SLC, ELC, SDF-1 and BLC/BCA-1) loose responsiveness to BLC/BCA-1, SLC and ELC while differentiating in plasma cells. The unresponsiveness for lymphoid CK is associated with the downregulation of their specific receptors CXCR5 and CCR7. On the contrary, CXCR4 expression is maintained, together with an increased sensitivity to SDF-1 (32). SDF-1 is highly expressed in bone marrow which is known to be a preferential plasma cell homing site. In addition,SDF-1 ectopic hyper-expression in the pancreas of transgenic animals has been shown to favour plasma cell local recruitment (33). These data together support the hypotesis that, at least in mice, SDF-1/CXCR4 may be critically involved in plasma cell trafficking regulation.

Antigen primed T cells, proliferate under the control of mature dendritic cells in the T cell area of secondary lymphoid organs and progressively differentiate into effector and memory cells. A recently proposed theory suggests that not all activated T lymphocytes loose secondary lymphoid organ homing habit. Recent studies have shown that a fraction of antigen primed T lymphocytes maintain their naïve-like CK receptor pattern (CCR7) and lymphoid homing properties. These cells (called “central memory” T cells) lack immediate effector capabilities which can be rapidly acquired upon secondary antigen stimulation. It has been suggested that “central memory” cells represent a clonally expanded antigen primed population which travels through secondary lymphoid organs, maintaining the immunologic memory for already experienced antigenic stimuli (34, 35). On the contrary, part of effector memory T lymphocytes, which display immediate effector functions, can down regulate lymphoid homing adhesion molecules and CKR (such as CCR7) and up regulate a series of glycoproteins, integrins and CKR specific for ligands produced in inflamed tissues (13, 34, 36, 37) where they can be recruited together with monocytes, immature dendritic cells and granulocytes. Some of this neo expressed molecules are shared between most of the lymphocyte subsets and give them a non-specific tropism for inflamed environments. However, other molecules synthesised upon activation can diversify the homing behaviour of effector T lymphocytes at two further levels. In particular, different migratory capabilities seem to be acquired by distinct populations with specific functional features (TH1 and TH2), and by cells activated in different anatomical compartments, giving rise to effector cells programmed to migrate selectively to specific tissues. CKR have a major role in these differentiation steps. Several studies have shown that Th1 and Th2 polarization is preferentially associated with distinct set of CKR: CXCR3 and CCR5 with TH1 whilst CCR4, CCR8 and CCR3 with Th2 cells (38-41). In this way, Th1 and Th2 polarized cells acquire distinct homing potential and can be specifically recruited by CK produced in tissues affected by specific types of inflammation in which their intervention is preferentially effective. Accordingly, different CK are differentially produced in distinct pathological situations. For example, Eotaxin/CCL11 (CCR3 ligand) is produced in tissues undergoing allergic inflammation, whilst IP-10/CXCL10, MIG/CXCL9, I-TAC/CXCL11 (CXCR3 ligands) are mainly produced in DTH reactions (13). However these observations remain controversial and while recent data have confirmed the preferential expression of some CKR by polarised T cell populations,they have also highlighted that all CKR can be expressed by either Th1 and Th2 populations (42). It is suggested that a more specific definition of differential CKR patterns by polarised T cells can be achieved only taking into account particular CKR combinations (42).

Alongside the non-specific tropism for inflamed tissues, effector memory T cells appear also to gain tissue specific homing properties, which are acquired under the influence of unknown factors acting during T cell priming in the corresponding draining lymphoid organs (43). The specificity of these subsets for their target organs derives from selective expression of specific adhesion molecules and CKR that recognise ligands that are differentially expressed at these tissue sites. To date, the best characterized tissue specific T cell populations are the skin and gut homing populations. Skin homing cells preferentially express CLA and CCR4 while gut homing lymphocytes distinctively express α4β7 and CCR9 that facilitate the adhesion and transmigration respectively on E-selectin/TARC and MAdCAM-1/TECK positive skin and intestinal microvasculature (44). Although indirect evidence for other tissues such as the synovial membrane has been provided (45, 46), no specific homing receptors or CK-driven migratory pathways have been so far identified.

Further complexity: Inflamed tissue draining lymph nodes and lymphoid neogenesis within chronic inflammatory sites

In previous paragraphs we have described naïve lymphocyte recirculatory routes between secondary lymphoid organs and the bloodstream and effector lymphocyte, monocyte, immature dendritic cell and granulocyte homing pathways to specific effector/ inflammatory sites. However, it should be emphasised that alternative recirculatory pathways may co-exist. For example, during an immuno/inflammatory response, monocyte and memory lymphocyte recirculation to secondary lymphoid tissues can be dramatically enhanced (47, 48). Although the process can be triggered by an increased cellular drainage from inflamed tissues through afferent lymphatic vessels, it also seems to involve an increased recruitment from the blood stream through high endothelial venules. Several pieces of evidence can explain, at a molecular level, this phenomenon. Inflammatory CK, expressed during inflammation in peripheral tissues and responsible for the local recruitment of monocytes and effector memory T lymphocytes, can be drained through afferent lymphatics to secondary lymphoid organs. Here, through fibroblastic reticular cell network conduits, they can be transported on high endothelial venules, favouring endothelial adhesion and vascular transmigration. This model suggests that secondary lymphoid tissues homing characteristics can be modulated remotely by factors produced by drained inflamed peripheral tissues (49). In addition, under immuno/ inflammatory conditions, the same secondary lymphoid organs have been shown to upregulate the production of many inflammatory CK, not expressed or expressed at very low level under resting conditions. The local production of these molecules, which can be exposed on high endothelial venules, can further contribute to monocyte and memory T lymphocyte local accumulation (50, 51) (Fig. 3 A, B).

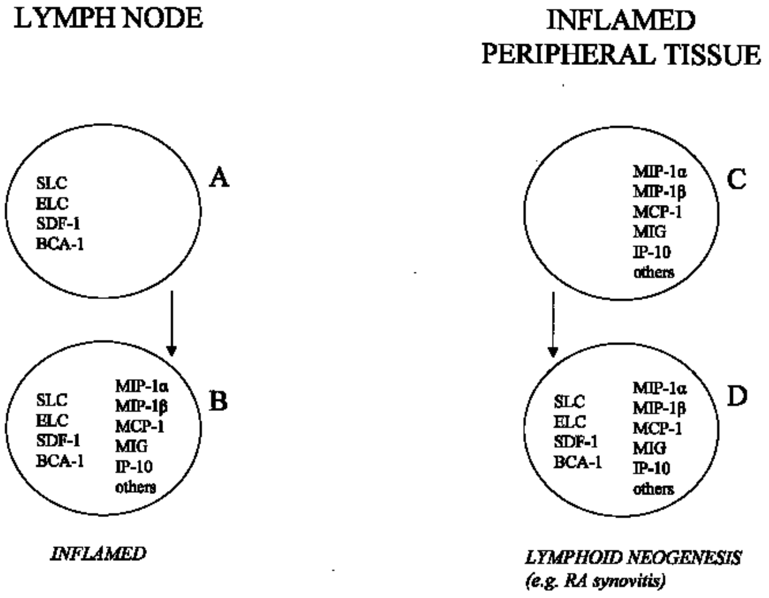

Fig. 3. (**A**) Secondary lymphoid organs constitutively express "lymphoid homing"CK which are functionally involved in naïve lymphocyte local homeostatic trafficking. (**B**) In inflamed lymph nodes some of the inflammatory CK can be locally produced and/or transported from periphery. (**C**) In inflamed peripheral tissues a number of "inflammatory" CK can be induced. These CK are involved in effector leukocyte local recruitment. (**D**) In long standing chronic inflammation,a process of lymphoid neogenesis can take place in involved tissue. This process is associated with the ectopic production of "lymphoid homing" CK.

On the other hand, it is well known that in chronic autoimmune inflammatory conditions such as rheumatoid synovitis or Sjogren's sialoadenitis a process of lymphoid neogenesis can take place in inflamed tissues (52-54). This phenomenon is frequently characterized by the development of secondary lymphoid organ-like cellular organizations with the constitution of discrete areas of T-B segregation, formation of FDC networks and even true germinal centres. In addition, in these areas, endothelial cells may show morphological and molecular features of the HEV present in secondary lymphoid organs. Interestingly, in association with this lymphoid-like organizational process, many lymphoid CK constitutively expressed in secondary lymphoid organs (such as SLC, ELC, BCA-1 and SDF-1) can be ectopically induced (55-58) (Fig. 3 C, D). In particular, in rheumatoid arthritis, the synovial expression of the CK SLC have been shown to be associated to the local presence of mature dendritic cells and naïve T lymphocytes (58, 59). Thus, the ectopic expression of lymphoid homing CK, might be involved in the 'misdirection' of naïve lymphocytes and mature dendritic cells from their route to secondary lymphoid organs to sites of chronic inflammation. In this context the inflamed tissue would acquire the functional features of a neo lymph node like station directly involved in lymphocyte priming and immune response construction.

Concluding remarks

CK regulate most of the dynamics involved in the immune system homeostasis, in the genesis of the immune response and the inflammatory process. This justifies the growing interests of rheumatologists and pharmaceutical companies for CK, as potential therapeutic targets. Although encouraging results have been obtained in mouse models of arthritis with CKR antagonists (60,61),many aspects of CK biology need further clarification for the definition of new, safe and effective, anti CK therapies in humans. In particular, the exact role of each single CK in the context of the inflammatory cascade has not been fully established, likewise their behaviour in the context of different pathological conditions. In addition, many CK are constitutively expressed in different organs and involved in vital processes such as lymphopoiesis and immune homeostasis making it problematic to target them therapeutically. Further insight is also necessary in the comprehension of the molecular aspect of CK-CKR interactions. As shown in Table I many different CK function as ligands for the same receptor and a single CK can bind to different receptors. Due to this high redundancy and versatility of CK and CKR, which probably has only been partially characterized, it is easy to imagine how post inhibition escape strategies could easily develop within the complex inflammatory network which characterise chronic immunomediated diseases. Furthermore, as recently evidenced, many CK not only function as agonists for their specific receptors but can behave as natural antagonists for other CKR, suggesting that the system is even more complex than it was believed so far and that the inhibition of one inflammatory pathway could lead to the amplification of collateral ones. In conclusion, data reported in this review highlight the enormous potential of CK as pharmacological targets for immunosuppression and underline the importance of basic research in the field of CK biology not only as a necessary requirement in prospective of new immunosuppressive therapies development but also for a better comprehension of rheumatic disease pathogenesis.

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