The chemokine SLC is expressed in T cell areas of lymph nodes and mucosal lymphoid tissues and attracts activated T cells via CCR7

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Secondary lymphoid-tissue chemokine, SLC, also known as exodus-2 and 6Ckine, is a novel CC chemokine with selectivity for T lymphocytes and preferential expression in lymphoid tissues. We have studied its production, receptor usage and biological activities. High levels of SLC mRNA were detected in lymph nodes, the gastrointestinal tract and several gland tissues, but no expression was found by Northern blot analysis in freshly isolated or stimulated blood monocytes and lymphocytes, or neutrophils and eosinophils. *In situ* hybridization revealed constitutive expression of SLC in the T cell areas and the marginal zone of follicles in lymph nodes and the mucosa-associated lymphoid tissue, but not in B cell areas or sinuses. Comparison with immunocytochemical staining showed similarity between the *in situ* expression of SLC and the distribution of interdigitating dendritic cells but not with sinus-lining dendritic cells, macrophages or T lymphocytes. SLC induced chemotaxis of T lymphocytes and its activity increased considerably when the cells were conditioned with IL-2 or phytohemagglutinin (PHA). Under optimal conditions SLC had unusually high efficacy and induced the migration of up to 50% of input T lymphocytes. SLC also induced Ca²⁺ mobilization in these cells. Similar responses were obtained with EBI1 ligand chemokine (ELC), and sequential stimulation with both chemokines led to cross-desensitization, suggesting that SLC acts via the ELC receptor, CCR7. This was confirmed using murine pre-B cells stably transfected with CCR7 which bound SLC with high affinity and showed chemotaxis and Ca²⁺ mobilization in response to both SLC and ELC. In T lymphocytes PHA and IL-2, which enhanced chemotactic responsiveness, also markedly enhanced CCR7 expression. In contrast to all known chemokine receptors, up-regulation of CCR7 by IL-2 was transient. A maximum was reached in 2–3 days and expression returned to initial levels within 8–10 days. The present study shows that SLC is constitutively produced within the T cell areas of secondary lymphoid organs and attracts T lymphocytes via CCR7.

**Key words:** Chemokine / Receptor regulation / T lymphocyte homing

1 Introduction

The number of chemokines and chemokine receptors have grown considerably within the past few years, and major progress has been made in the understanding of chemokine-induced mobilization of T lymphocytes [1]. In these cells expression of chemokine receptors is usually influenced by stimulation. CCR1, CCR2, CCR5 and CXCR3, for instance, are barely detectable in resting T lymphocytes but are markedly up-regulated by IL-2 [2–4]. CXCR4, on the other hand, is expressed on naive as well as activated T lymphocytes [3, 5, 6]. Some more recently described receptors, like CCR6 [7], CCR7 [8] and CXCR5 [9], are prominent in lymphocytes and recognize chemokines that are constitutively expressed in lymphoid tissues [8–12]. They are of particular interest for their potential role in lymphocyte homing, which could be controlled by chemokines such as EBI1 ligand chemokine (ELC)/MIP-3β [8, 11], LARC/MIP-3α/exodus

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**Abbreviations:**

ELC: EBI1 ligand chemokine [Ca²⁺];
Intracellular Ca²⁺ concentration
SLC: Secondary lymphoid-tissue chemokine
MALT: Mucosa-associated lymphoid tissue

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[10–12], BCA-1 [9], DC-CK1/PARC [13, 14] as well as secondary lymphoid-tissue chemokine (SLC)/6Ckine/exodus-2 [15–17] as described in the present paper. While most chemokines of this type act preferentially on T lymphocytes, BCA-1 is a selective attractant for B lymphocytes. It is highly expressed in secondary lymphoid tissues and is the ligand for the former orphan receptor BLR1/MDR15 [18, 19] which is now called CXCR5 [9]. B cell colonization is impaired in BLR1/CXCR5-deficient mice [20], suggesting that chemotactic recruitment by locally produced BCA-1 regulates the homing of B lymphocytes.

In this report we show that SLC is constitutively expressed in T cell areas of lymph nodes and the mucosa-associated lymphoid tissue (MALT) system, is chemotactic for activated T lymphocytes and selectively binds CCR7, a receptor that also recognizes ELC [8]. Owing to these properties, SLC could direct the homing of T lymphocytes that are engaged in antigen recognition and clonal expansion.

2 Results

2.1 SLC is constitutively expressed in secondary lymphoid tissues and the MALT

SLC was recently reported as a new CC chemokine with selectivity for T lymphocytes [15–17]. To understand its role in T cell migration, we initially studied the expression of SLC by Northern blotting in several human blood cells and cell lines that were stimulated in different ways. Freshly isolated blood monocytes, neutrophils, eosinophils and lymphocytes did not contain detectable levels of SLC mRNA. Monocytes stimulated by pro-inflammatory cytokines (IL-1β, IFN-γ and TNF-α), endotoxin or adherence, which are a rich source of various chemokines [21], were equally negative. In addition, no SLC transcripts were detected in lymphocytes stimulated with IL-2, PHA or anti-CD3 + anti-CD28, cultured human umbilical vein endothelial cells stimulated with IFN-γ or TNF-α, and the lung epithelial cell line A549 stimulated with IFN-γ, TNF-α or endotoxin. By contrast, hybridization signals corresponding to a single RNA species of 0.9 kb as described by Nagira et al. [15] were detected with RNA from intestinal tissue (data not shown). Expression was also assessed by RNA dot-blotting in a large collection of normal human tissues. As shown in Fig. 1, exceptionally high expression of SLC was detected in lymph nodes and the appendix, and marked expression was observed in the gastrointestinal tract and several gland tissues which is in keeping with the designation of SLC as secondary lymphoid-tissue chemokine [15].

![Figure 1](image-url)  
**Figure 1.** Constitutive SLC expression in human tissues. An RNA dot-blot displaying normalized amounts of poly(A) RNA from a wide range of adult and fetal human tissues was probed with 32P-SLC DNA. Relative levels of SLC mRNA expression are presented. The maximum value (100 %) corresponded to 19 × 10⁶ relative integrated counts obtained with RNA from lymph node tissue. Analysis was performed with a phosphorimager (Molecular Dynamics Inc., Sunnyvale, CA) and the ImageQuant software. Expression levels below 2 % (brain, spinal cord, skeletal muscle, pituitary gland, testis, liver, placenta, fetal kidney, liver and brain) are not shown.

2.2 In situ hybridization

To determine the distribution and cellular origin of SLC, in situ studies were performed on lymphoid tissues. Fig. 2A shows bright and dark field photographs of lymph node sections hybridized with sense and antisense probes. The antisense probe detected high numbers of SLC-expressing cells in the marginal zone of follicles and throughout the interfollicular tissue, while the B cell areas of the follicles and the sinuses were negative. No hybridization was obtained with the sense probe, validating the observed distribution of SLC expression. Serial sections were then analyzed by in situ hybridization for SLC expression and by immunocytochemistry with mAb recognizing T lymphocytes (CD3), mononuclear phagocytes (CD68), interdigitating (CD1a) and sinus-lining (Ki-M9) dendritic cells. As indicated by Fig. 2B, the SLC-producing cells are confined to the T cell areas but their distribution is distinct from that of CD3-positive cells. The staining with anti-CD68 and the morphological identification of high endothelial venules (not shown) suggest that SLC is not produced by mac-
Figure 2. *In situ* expression of SLC in lymph nodes. (A) Hybridization of sections of lymph follicles with SLC antisense (a, b) and sense (c, d) probe. Bright and dark field photographs, with positive staining as black and white granules, respectively, are shown. Cell nuclei counterstained with hematoxylin appear in blue. (B) Serial sections of follicles (a–d and e–h). Hybridization with SLC antisense probe (a and e, dark field) showing SLC expression in the marginal zone (arrow) of the follicle (F), and the interfollicular, diffuse lymphatic tissue, but not in the sinuses (S). Immunocytochemical staining (red color) is shown for interdigitating dendritic cells (b, anti-CD1a), sinus-lining dendritic cells (f, Ki-M9), T lymphocytes (c and g, anti-CD3) and macrophages (d and h, anti-CD68).

Rophages or endothelial cells. Staining with anti-CD1a and the mAb Ki-M9 [22] clearly discriminates between interdigitating and sinus-lining dendritic cells, and shows that the distribution of SLC-expressing cells coincides with that of interdigitating dendritic cells. *In situ* hybridization performed on appendix sections revealed a similar pattern of SLC expression.
Figure 3. *In vitro* chemotaxis in response to SLC. Migration of blood T lymphocytes, monocytes and neutrophils in response to SLC (○), SDF-1 (□) and IL-8 (△). Prior to the chemotaxis assay, blood T lymphocytes were cultured in RPMI 1640 medium with 5% human serum either overnight without additions or for 4 days in the presence of IL-2 [2]. Monocytes and neutrophils were used immediately after isolation. Numbers of migrated cells (number of adherent cells ± SD per five high-power fields in triplicate assays) are shown. The data are representative for two (monocytes, neutrophils) and four (T lymphocytes) independent experiments with cells from different donors.

2.3 SLC attracts leukocytes

SLC induced chemotaxis of freshly isolated blood lymphocytes and monocytes *in vitro* (Fig. 3). Its potency was moderate since concentrations of at least 100 nM were required for lymphocytes, and modest migration of monocytes was observed at 1 μM only. However, when T lymphocytes were cultured in the presence of IL-2, the effect of SLC increased considerably both in efficacy and potency. It was reported by others that SLC induces lymphocyte chemotaxis [15–17].

2.4 SLC is a functional ligand for CCR7

SLC induced changes in intracellular Ca²⁺ concentration ([Ca²⁺]i) in IL-2 conditioned T lymphocytes. Similar responses were obtained with ELC, and sequential stimulation with both chemokines led to cross-desensitization, which is indicative of receptor sharing (data not shown). Since ELC was reported to act via CCR7 [8], we prepared cells expressing this receptor to study the effects of SLC. DNA containing the coding region for CCR7 was generated by PCR, and five independent clones were sequenced to completion. Surprisingly, all five clones differed from the reported CCR7 sequence [23] at position 607–612 (ATC TGG instead of AGC GCC) yielding an isoleucine and a tryptophan instead of serine and alanine in transmembrane domain 4. This tryptophan is highly conserved among chemokine receptors [1]. A further difference was at position 1072 (C replaced by A), yielding an isoleucine instead of a leucine in the C-terminal domain. The CCR7 DNA was transfected into the mouse pre-B cell line 300-19, and cell clones that stably expressed CCR7 mRNA were used for functional assays. As shown in Table 1, of 32 human chemokines and some additional peptide agonists, only ELC, SLC and SDF-1 induced [Ca²⁺]i changes in CCR7-expressing 300-19 cells (Fig. 4a, b). In contrast to SLC and ELC, SDF-1 also induced a response in the parental 300-19 cells, which express CXCR4 [24, 25]. The interaction of
SLC and ELC with CCR7 is of high affinity, as indicated by the rapid rise in \([\text{Ca}^{2+}]\), observed at nanomolar chemokine concentrations. Full cross-desensitization between ELC and SLC was observed (Fig. 4c), confirming that both chemokines act on CCR7 with similar affinity. As shown in Fig. 4d, 300-19 cells expressing CCR7 specifically bind \(^{125}\text{I}-\text{SLC}.\) In several experiments, nonspecifically bound material amounted to 58–71 % of the total cell-associated radioactivity, and this value could not be decreased by alternative radioiodination protocols or changes in the binding conditions. No specific binding was observed with parental 300-19 cells. Scatchard analysis revealed \(2 \times 10^4\) SLC binding sites per 300-19 cell with a single dissociation constant (Kd) of 1.6 nM.

### 2.5 CCR7-expressing cells migrate in response to SLC

The chemotactic activity of SLC and ELC was compared. Fig. 5 shows that both chemokines are highly effective on 300-19 cells expressing CCR7 and are inactive on the parental cells. The response to both chemokines was virtually identical and characteristically bimodal. The activity threshold was around 30 nM, and maximum migration was obtained for both chemokines between 100 and 300 nM.

### 2.6 Receptor expression and functional responses of T lymphocytes

It was previously shown that treatment with IL-2 markedly enhances the expression of CCR1, CCR2, CCR5 and CXCR3 and the ability of T cells to migrate in response to chemokines recognizing these receptors [2–4]. By analogy, we found that IL-2 enhances the chemotactic efficacy and potency of SLC for T lymphocytes (Fig. 3). Several stimulatory conditions were, therefore, adopted to study the regulation of CCR7 expression and responsiveness to SLC. As shown in Fig. 6a, freshly prepared blood T cells expressed low but detectable levels of CCR7 which was rapidly up-regulated by treatment with PHA or IL-2. Induction of CCR7 mRNA was evident as early as 8 h (not shown) and reached a maximum 3 days after addition of PHA. A similar time course was
observed after stimulation with IL-2: peak levels were reached in 2–3 days, but then expression decreased to background levels within about 8 days of culture. The behavior of CCR7 differs greatly from all known chemokine receptors as represented by CCR2, which is also induced by IL-2, but rises much more slowly and is not up-regulated by PHA, confirming previous observations [2].

Similar changes were observed for T lymphocyte chemotaxis in response to SLC. As shown in Fig. 6b, SLC-induced migration rose rapidly to high levels under both stimulatory conditions and then decreased, as shown for the treatment with IL-2. The maximum was reached after about 3 days, and migration returned to the initial levels after 8–12 days of culture (not shown). A slight enhancement to about 10% of migrating T lymphocytes was obtained within 3 days by culturing with medium alone or in the presence of anti-CD3 and anti-CD28. In agreement with the expression of CCR2, MCP-1-dependent migration increased slowly and steadily as reported previously [2–4]. Ca²⁺ mobilization, another indication for chemokine function, was barely detectable in freshly isolated lymphocytes at SLC concentrations up to 100 nM. It increased to a maximum after 4 days in the presence of IL-2, and declined to the initial levels within 10 days (data not shown).

3 Discussion

SLC [15], also termed 6Ckine and exodus-2 [16, 17], belongs to a recently recognized class of CC chemokines which are expressed preferentially in lymphoid tissues and are chemotactic for lymphocytes. The present study shows that this chemokine is highly expressed in T cell areas of the interfollicular compartment and the marginal zone of follicles but not in the germinal centers and sinuses. No expression, by contrast, was observed in resting or activated T lymphocytes, monocytes or macrophages. Comparison of the pattern of SLC expression by in situ hybridization in lymph nodes and the distribution of lymphocytes, mononuclear phagocytes as well as sinus-lining and interdigitating dendritic cells by immunocytochemistry in serial sections suggests that SLC is produced by interdigitating dendritic cells. SLC expression is constitutive rather than induced, since high levels of the corresponding RNA were detected in normal human tissues. In view of its restricted, constitutive expression in T cell areas and its high efficacy as T lymphocyte attractant, SLC may regulate the homing of T lymphocytes into secondary lymphoid organs. There is some analogy between SLC and DC-CK1/PARC which is produced by dendritic cells in T cell areas [13]. However, SLC is not expressed in germinal centers and thus has a more restricted tissue localization.

Identification of CCR7 as the receptor for SLC was crucial for the study of leukocyte responses to this chemokine. Previous studies have shown that CCR7 [8], formerly known as EBI1 [23] or BLR2 [26], is preferentially expressed in T lymphocytes after stimulation with mitogens or anti-CD3, or in B cells infected by EBV. Here we show that fresh blood T lymphocytes have low levels of CCR7 transcripts, but rapidly up-regulate this receptor in response to PHA and IL-2. CCR7 induction is rapid and transient and differs markedly from that of several receptors, up-regulated by IL-2, like CCR1, CCR2, CCR5 and CXCR3 [2–4] which is protracted and related to expansion of activated T lymphocytes that are recruited to sites of inflammation.

SLC is a chemoattractant of unusual efficacy, as indicated by the migration of up to 50% of input T lympho-
cytes under conditions of optimal receptor expression, and of even higher percentages of transfected pre-B cells expressing CCR7. Comparable levels of efficacy are rare for chemokines, and were observed with SDF-1 [27] and BCA-1 [9] using T and B lymphocytes, respectively. By comparison, MCP-1 which is regarded as a major attractant for T lymphocytes [2, 28, 29], is much less effective. On the other hand, as indicated by the concentrations at which maximal migration is observed, SLC, SDF-1 and BCA-1 are much less potent than MCP-1. The difference may be related to the type of function. High efficacy and low potency may be characteristic for chemokines like SLC, SDF-1 and BCA-1 that are involved in lymphocyte homeostasis, while high potency with lesser efficacy may be more appropriate for chemokines functioning in inflammation. Northern blot analysis showed that the SLC receptor, CCR7, is restricted to T lymphocytes. The observed migration of monocytes at high SLC concentrations may be due to low-affinity interaction of the ligand with another chemokine receptor.

Of particular interest is the transient effect of T lymphocyte activation on CCR7 expression and receptor-dependent responses to SLC and, by analogy, ELC which uses the same receptor. The difference in time course and stimulus dependency of receptor expression clearly indicates that SLC and ELC on the one hand and inflammatory-related chemokines, e.g. the MCP, RANTES, MIP-1α, MIP-1β, IP10 and Mig, on the other hand, target different populations of T lymphocytes. In fact, while responses to MCP-1, RANTES and other chemokines arising in inflammation are restricted to T cells of memory phenotype (CD45RO+), no overt difference in the response to SLC was observed between memory and naive (CD45RO– and CD45RA+) T lymphocytes isolated from fresh blood (data not shown).

Taken together, our data suggest that interdigitating dendritic cells within lymph nodes and the MALT system constitutively produce the CC chemokine SLC, which attracts T lymphocytes via CCR7. In view of the low level of CCR7 expression in resting T lymphocytes and its rapid and transient up-regulation by PHA and IL-2 it is conceivable that SLC acts preferentially on T lymphocytes which are already present in the T cell areas of lymph nodes. T cells that are engaged in antigen recognition and thus have been exposed to short-term activation signals, which can lead to CCR7 expression, may be the true target for SLC and ELC. The chemotactic effect of these chemokines may well result in the trapping of antigen-specific cells to avoid their premature egress from a T cell area. Since CCR7 expression is transient, clonally expanded effector T lymphocytes can eventually leave secondary lymphoid organs and participate in inflammatory responses. The trapping of mobile cells by chemoattractants could be important in several aspects of lymphocyte homing and development. It has been suggested that chemokines released by dendritic cells may ensure the encounter and coalescence with naive T lymphocytes [13] and that SDF-1 may favor the proliferation of B cell precursors by attracting and trapping them in close contact with bone marrow stromal cells [24].

4 Materials and methods

4.1 Cytokines

SLC, ELC and reference chemokines were chemically synthesized according to established protocols [30]. Recombinant human IL-2 was generously provided by Dr. A. Lanaveccia (Basel Institute for Immunology, Basel, Switzerland).

4.2 Antibodies and cells

mAb to CD3, CD68, and CD1a for immunocytochemical staining were from Dako Corp., Carpinteria, CA. The mAb Ki-M9 detecting sinus-lining dendritic cells [22] was kindly provided by Dr. R. Parwaresch (Institute for Hematopathology, University of Kiel, Germany). Human neutrophils [31], monocytes [32] and lymphocytes [2] were isolated from donor blood buffy coats. Where indicated, PBL were activated by culturing in the presence of IL-2 or PHA [2].

4.3 CCR7-transfected 300-19 cell clone

CCR7/EBI1 cDNA was generated by PCR with the primers CCR7-SE and CCR7-AS corresponding to the nucleotide sequence of EBI1 [23] and cDNA from IL-2-expanded T lymphocytes as amplification template [9, 25]. The primers CCR7-SE, 5’-TTTAGATCCGAGACCGTCTAGGACC, and CCR7-AS, 5’-TTAGGATCCGAGACCGTCTAGGACC, correspond to positions 52–70 and 1201–1219 in the published sequence [23] and span the entire coding region for CCR7 of 378 amino acids. PCR amplifications were performed in total volumes of 30 μl, containing 1× PCR buffer including 1.5 mM MgCl2 (Gibco-BRL Life Technologies Inc., Paisley, GB), 2 μl of cDNA preparation, 0.4 μM primers and 280 μM dNTP. To start DNA synthesis, 1.5 U Taq polymerase (Gibco-BRL) were added after cDNA denaturation and allowed to progress for 30 cycles (1 min, 57 °C; 45 s, 72 °C; 15 s, 95 °C) in a master cycler 5330 (Eppendorf-Netheler-Hinz Inc., Hamburg, Germany). The amplified DNA was purified, digested with EcoRI and BamHI and subcloned into the mammalian expression vector SRpuro (provided by Dr. F. Arenzana-Seisdedos, Pasteur Institute, Paris, France) and five independent clones were sequenced to completion. To generate stable transfectants, 5 × 10⁴ 300-19 mouse pre-B cells in 400 μl electroporation buffer were transfected with
20 μg linearized Srpuuro-CCR7 plasmid DNA as described [33]. Puromycin-resistant 300-19 clones were established by limited dilution cultured in the presence of 1.5 μg/ml puromycin (Sigma Chemical Co., St Louis, MO) and screened for CCR7 expression by RNA dot-blot analysis.

4.4 Ca\(^{2+}\) responses to chemokines

Changes in [Ca\(^{2+}\)] were measured in cells loaded with fura-2 by incubation for 25 min at 37 °C with 0.1 nmol fura-2/AM per 10\(^6\) cells in a buffer containing 136 mM NaCl, 4.8 mM KCl, 1 mM CaCl\(_2\), 5 mM glucose and 20 mM Heps, pH 7.4. After centrifugation, loaded cells were resuspended in the same buffer (2 × 10\(^6\) cells in 800 μl), stimulated with the indicated chemokine at 37 °C, and the [Ca\(^{2+}\)]-related fluorescence changes were recorded [34].

4.5 In vitro chemotaxis

Cell migration was assessed in 48-well chambers [4]. RPMI 1640 supplemented with 20 mM Heps, pH 7.4, and 1 % pasteurized human plasma protein solution (Swiss Red Cross Laboratory, Bern, Switzerland) was used to dissolve the chemokines and to resuspend the cells. The duration of the assay was 120 min for T lymphocytes, CCR7 transfectants and parental 300-19 cells, and 25 min for neutrophils and monocytes. Migrated cells were counted in the lower well and/or on the lower side of the filters at 1000× magnification in five randomly selected fields.

4.6 Expression of SLC and chemokine receptors

SLC expression was examined in a dot-blot analysis of a human Immune System Multiple Tissue Blot II™ (Clontech, Palo Alto, CA). A \(^{32}\)P-labeled SLC DNA (370-bp PstI fragment, position –2 to 368 of the published sequence [15] comprising the N-terminal coding region, 107 cpm/μg) was used as hybridization probe at 5 × 106 cpm/ml hybridization solution. For receptor expression studies, total RNA was extracted from T lymphocytes immediately after isolation from the blood and after culture for different times in the presence of 1 mg/ml PHA or 200 U/ml IL-2, and 10-μg samples were analyzed by Northern blotting using \(^{32}\)P-labeled receptor DNA inserts (109 cpm/μg DNA) as hybridization probes [2, 4].

4.7 \(^{125}\)I-SLC binding to 300 19-CCR7 transfectants

Synthetic SLC was iodinated with Enzymobeads (Bio-Rad, Hercules, CA) [35]. The iodinated chemokine was separated from free iodine by gel filtration chromatography (Bio-Gel P-6 DG, Bio-Rad), and the bindability was calculated and found to be 25 %. Binding of \(^{125}\)I-SLC to 300-19 transfec-
tants expressing CCR7 and analysis of binding data were performed as previously described [25, 35].

4.8 In situ hybridization

Lymph node cryosections (5 μm) were prefixed in 4 % paraformaldehyde and pretreated with 1 μg/ml proteinase K (30 min 37 °C in 0.1 M Tris-HCl, pH 8.0, containing 50 mM EDTA), postfixed in 4 % paraformaldehyde, dehydrated through graded alcohol and acetylated by acetic anhydride (10 min at room temperature in 0.1 M triethanolamine, pH 8.0). The dehydrated sections were then hybridized overnight at 45 °C with sense or antisense \(^{35}\)S-labeled SLC RNA probes (106 cpm/section) consisting of a 370-bp fragment of the N-terminal coding region, generated by in vitro transcription (Boehringer Ltd., Mannheim, Germany). Specificity of binding was ensured by three stringent washes (45 min 54 °C in 50 % formamide, 1 mM EDTA, 10 mM DTT, 2× SSC) and a single-strand RNA digest using RNase T1 and A (30 min 37 °C, 1 U/ml RNase in 2× SSC, 1 mM EDTA, pH 8.0). The hydrated sections were dipped in Kodak photographic emulsion NTB-2 and exposed for 10 days. Development and fixation were performed according to the Kodak protocol. The labeled sections were counterstained with hematoxylin and embedded in Aquamount.

4.9 Immunohistochemistry

Cryostat tissue sections were fixed and incubated overnight with one of the following mAb at a 1:50 dilution: anti-CD3, anti-CD68, anti-CD1a (Dako Corp., Carpinteria, CA), or Ki-67 (a generous gift of Dr. R. Parwaresch, Institute for Hematopathology, University of Kiel, Germany) which recognizes sinus-lining dendritic cells [22, 36]. As controls, species- and isotype-matched Ig (Sigma Chemical Co., St. Louis, MO) were used. After washing, the slides were incubated for 30 min with biotin-labeled sheep anti-mouse Ab (Amersham, Arlington Heights, IL) at 1:800 dilution. After additional washes, the slides were incubated for 30 min with streptavidin-biotin-alkaline-phosphatase (Dako Corp., Carpinteria, CA). All incubations were performed at room temperature. The color reaction was developed with New Fuchsino (Dako Corp.). Slides were counterstained with Mayer’s hematoxylin and analyzed with a Leitz Dialux 20EB microscope.

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5 References


