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Biochemical and Biophysical Research Communications 304 (2003) 36–40
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# Antigen-induced chemokine activation in mouse buccal epithelium

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Received 3 March 2003

**Abstract**

The oral mucosa is an active though poorly understood immunological site. Using an experimental animal system involving antigen priming into the oral mucosa of transgenic mice expressing T cell receptor (TCR) for a peptide antigen of hen-egg lysozyme (HEL), the expression of six chemokine receptors and seven chemokine ligands were studied before and after antigen exposure. Within 24 h of local antigen priming, the expression of three chemokine receptor genes (CCR3, CCR5, and CCR7) and three chemokine ligand genes (CCL12, CCL19, and CCL25) were significantly upregulated. These included chemokines known to be responsible for the trafficking of T cells and other leukocytes into tissue sites. Additionally, expression of the chemokine ligand gene, CCL25 (thymus-expressed chemokine [TECK]), which has been linked to T cell migration and/or local T cell development in the intestine, was also markedly elevated in buccal epithelia after antigen exposure. These findings define a process of selective activation of proinflammatory chemokines and/or their receptors following local antigen exposure, and they provide the first evidence, indicating that this may be accompanied by in situ development of T cells in oral tissues.


*Keywords:* Oral; Mucosa; Antigen priming; T cells; Hematopoietic

The oral mucosa is continually confronted with a vast number of pathogens and antigens, and is susceptible to iatrogenic inflammation and hypersensitivity resulting from dental treatment and therapy. Yet, compared to the spleen or lymph nodes, considerably less is known about the mechanisms that control the adaptive, innate, or inflammatory immune response in the mouth. In general, the oral mucosa is known to have CD4 and CD8 T cells [1–3], and similar to other mucosal sites [4,5], these consist of both T cell receptors (TCR)$\alpha\beta^+$ and TCR$\gamma\delta^+$ T cells [6]. Increases in the numbers of $CD8^+$ T cells in the oral mucosa have been noted in disease conditions such as recurrent aphthous ulceration [3]. Other types of mononuclear cells, in particular macrophages, Langerhans' cells, dendritic cells, and mast cells [7–12], as well as polymorphonuclear leukocytes [10,11] are also normally present in significant numbers in the oral mucosa, or are recruited in following antigen deposition locally. Moreover, in various pathological conditions such as with oral submucous fibrosis, cytokine levels of proinflammatory and immunoregulatory cytokines can be significantly elevated [2]. IL-6, IL-8, and prostaglandin $E_2$ are elevated to varying levels following in vitro exposure of human buccal epithelial cells to nickel, cobalt, palladium, and triethylene glycol dimethacrylate all of which are frequently used in dental materials [13]. These observations collectively point to a highly active and dynamic immunological response in the oral mucosa.

Chemokines comprise a group of small molecular weight peptides that are used to initiate or control a wide variety of immunological and biological activities, including hematopoietic cell trafficking [14,15], angiogenesis [16], and tumor control [17–19]. These molecules operate through the regulated expression of specific chemokine receptors on cells of the immune system or tissue cells, and through the elaboration of chemokines (chemokine ligands) targeted to those receptors. Many chemokines have been shown to play a prominent role in the inflammatory process [20], and they are themselves influenced by other inflammatory molecules such

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doi:10.1016/S0006-291X(03)00533-3

as IL-1, TNFα, and IFN-γ (reviewed in [21]). A recent study from our laboratory demonstrated a process of dynamic immune regulation of TCRγδ$^+$ T cells in the buccal epithelium of mice following local priming with either bovine serum albumin (BSA) or influenza virus [22]. That study resulted in the novel observation that antigen priming directly into buccal tissues causes a transient loss of TCR variable (V)γ gene expression, with subsequent recovery in expression occurring by day 4 post-antigen exposure [22]. Coupled with the overall lack of information regarding the immune-regulating properties within oral tissues, those findings prompted us to explore the involvement of chemokines in the buccal epithelium during an acute immune response to antigenic stimulation. The experimental system selected for this involved the use of transgenic mice expressing a TCR heterodimer specific for peptide sequence 44–61 of the hen-egg lysozyme (HEL) molecule [23]. This model permitted us to examine the immune response in buccal tissues in a system in which all T cells were focused on the same nominal antigen. As shown here, using a panel of 13 chemokine ligands/chemokine receptors, we demonstrate: (i) that the expression of chemokine and/or chemokine receptor genes occurs rapidly following local antigen exposure, (ii) that chemokine ligand and/or receptor gene activation is highly selective, and (iii) that the thymus-expressed chemokine (TECK) is upregulated and expressed at high level following HEL priming, suggesting a possible process of local T cell development in the oral cavity similar to that known to occur in the intestinal tract.

## Materials and methods

*Mice.* Breeding pairs of transgenic mice expressing a major histocompatibility class II complex-restricted TCR for HEL (STOCK TgN[TcrHEL3A9]Mmd), hereafter referred to as 3A9 mice, were purchased from The Jackson Laboratories, Bar Harbor, ME. Animals were bred at the University of Texas Dental Branch, Houston, TX.

*Animal priming, tissue isolation, and RT-PCR analyses.* Mice were anesthetized by isofluorane inhalation (IsoFlo; Abbott Laboratories; North Chicago, IL) and injected with 100 μl of 10 mg/ml HEL (Sigma Chemicals; St. Louis, MO) suspended in PBS. Animals were sacrificed 24 h later and the buccal epithelium was isolated using published techniques [22]. In brief, cheek tissue pieces were removed and cut from the underlying connective tissues under a dissecting microscope. Tissue pieces were incubated at 37 °C for 2.5 h in $Ca^{2+}/Mg^{2+}$ free PBS containing 20 mM EDTA. Epithelial sheets were removed and RNAs were isolated using a 4PCR Kit #1914 (Ambion; Austin, TX). cDNAs were prepared with an RT-PCR Kit #1402-2 (Clontech; Palo Alto, CA). Primer sequences used were:

| | | |
|---|---|---|
| CCR2: | forward | 5′-CCTGCAAAGACCAGAAGAGG-3′ |
| | reverse | 5′-TCCAAGCTCCAATTTGCTTC-3′ |
| CCR3: | forward | 5′-GCAAAATGCTGTCTGGGTTT-3′ |
| | reverse | 5′-CCTCTGGATAGCGAGGACTG-3′ |
| CCR5: | forward | 5′-CACTGCTGCCTAAACCCTGT-3′ |
| | reverse | 3′-TTCCTACTCCCAAGCTGCAT-3′ |
| CCR6: | forward | 5′-CCTACCGTTCTGGGCAGTTA-3′ |
| | reverse | 5′-AGGGCTTGAGATGATGATGG-3′ |
| CCR7: | forward | 5′-CGGAGATTCAAGGACAGAGC-3′ |
| | reverse | 5′CAGCAGGGTCACCTTCTCTC-3′ |
| CCR9: | forward | 5′-TGACTCCACTGCTTCCACAG-3′ |
| | reverse | 5′-CAGAAGGGAAGAGTGGCAAG-3′ |
| CCL5: | forward | 5′-ATATGGCTCGGACACCACTC-3′ |
| | reverse | 5′-GGGAAGCGTATACAGGGTCA-3′ |
| CCL9: | forward | 5′-ACAGCAAGGGCTTGAAATTG-3′ |
| | reverse | 5′-TTGTAGGTCCGTGGTTGTGA-3′ |
| CCL11: | forward | 5′-TGACACTAACCCAGAGCCTAAG-3′ |
| | reverse | 5′-CATAATGACTTCCAGTCCCATC-3′ |
| CCL12: | forward | 5′-CTTCTATGCCTCCTGCTCATAGC-3′ |
| | reverse | 5′-CGGACGTGAATCTTCTGCTTAAC-3′ |
| CCL19: | forward | 5′-TCTCCTCCCTCCCCTTAGAA-3′ |
| | reverse | 5′-CGGCTTTATTGGAAGCTCTG-3′ |
| CCL20: | forward | 5′-CGTCTGCTCTTCCTTGCTTT-3′ |
| | reverse | 5′-AGGAGGTTCACAGCCCTTTT-3′ |
| CCL25: | forward | 5′-GTGATGATGCCCAGAAAGACC-3′ |
| | reverse | 5′-TCAGCAATCATCAATAGCCAATAG-3′ |
| β-actin | forward | 5′-ATGGATGACGATATGGCTG-3′ |
| | reverse | 5′-ATGAGGTAGYCTCTAAGGT-3′ |

Amplification consisted of 40 cycles at 95 °C for 1 min, 59 °C for 1 min, and 72 °C for 1 min using a Biometra T-Gradient thermocycler (Whatman Biometra; Gottingen, Germany). For semi-quantitative RT-PCR, 1:3 serial dilutions of cDNA were made prior to amplification. PCR products were run on a 2% agarose gel.

## Results

### *Regulated chemokine gene expression in 3A9 mice following buccal antigen priming*

3A9 mice were injected into the buccal mucosa with HEL as described in the Materials and methods. Twenty-four hours after priming, mice were sacrificed, buccal epithelia were recovered, and tissues were analyzed by RT-PCR for expression of 13 chemokines receptors or chemokine ligands as shown in Table 1. Findings were particularly notable in that there was a spectrum of chemokine receptor or chemokine ligand expression that consisted of three patterns. First, two chemokine ligands (CCL5 and CCL9) were expressed at low levels prior to antigen priming and did not increase after priming. Second, five chemokine receptors or chemokine ligands were expressed at high levels both prior to and after antigen priming; these were CCR2, CCR6, CCR9, CCL11, and CCL20. Third, six chemokine receptors or chemokine ligands were significantly upregulated within 24 h of antigen priming; these were CCR3, CCR5, CCR7, CCL12, CCL19, and CCL25. A summary of these findings is shown in Table 1.

To confirm the changes in chemokine receptors or chemokine ligands that had increased following HEL

Table 1
Differential expression of chemokine receptors and chemokine ligands in buccal epithelia of 3A9 mice following local HEL priming

| Chemokine receptor (R)/ligand (L) | Expression before priming | Expression after priming |
|---|---|---|
| CCR2 | High | High |
| CCR3 | Negative | High[a] |
| CCR5 | Low | High[a] |
| CCR6 | High | High |
| CCR7 | Negative | High[a] |
| CCR9 | High | High |
| CCL5 | Low | Low |
| CCL9 | Negative | Negative |
| CCL11 | High | High |
| CCL12 | Low | High[a] |
| CCL19 | Neg/low | High[a] |
| CCL20 | High | High |
| CCL25 | Low | High[a] |

[a] Increase in chemokine receptor or chemokine ligand gene expression 24 h after intra-buccal antigen priming.

priming, semi-quantitative RT-PCR analyses were done as shown in Fig. 1. Note that by 24 h after intra-buccal HEL priming of 3A9 mice there was at least a 9-fold increase in gene expression of the chemokine receptors and ligands identified in Table 1. This included three chemokine receptor groups: CCR3 (the receptor for Eotaxins-1, 2, and 3, MCP-2, 3, and 4, and RANTES), CCR5 (the receptor for MIP-1 and RANTES), and CCR7 (the receptor for MIP-3β/ELC and 6CKine/LC). Additionally, gene expression of CCL12 and CCL19 were significantly elevated by 24 h after HEL priming. Collectively, these form a complex pattern of chemokine activation in the buccal epithelium following local antigen exposure.

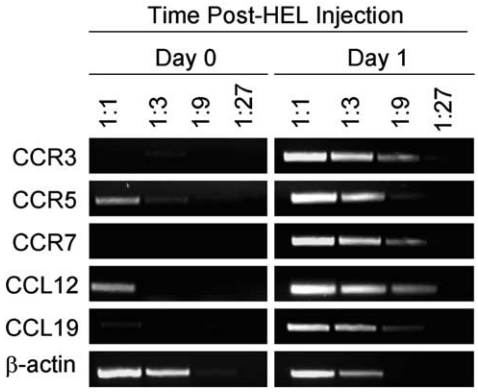


Fig. 1. Semi-quantitative RT-PCR analyses of chemokine receptors CCR3, CCR5, and CCR7, and chemokine ligands CCL12 and CCL19 from buccal epithelium of 3A9 mice following local HEL antigen priming. Note the increase in gene expression within 24 h of antigen exposure.

*The buccal epithelium constitutively expresses CCR9 and CCL25, and the expression of CCL25 is upregulated after local antigen priming*

The initial screening of chemokine/chemokine receptors described in Table 1 revealed the unexpected finding that the receptor for CCL25 was expressed at high levels in the buccal epithelium of both non-antigen-primed and antigen-primed mice. However, CCL25, the chemokine ligand for CCR9, was expressed at low levels in normal 3A9 mice but was significantly upregulated after HEL priming. These findings were of particular interest since CCL25 has been shown to have a highly selective distribution in its expression, so far limited to the thymus and the intestine [24]. Additionally, that chemokine is believed to be responsible for regulating the development of T cells in the thymus and for the local development of T cells in the intestine [24,25]. Using semi-quantitative RT-PCR analyses, expression of CCL25 was found to have a 3-fold increase in 3A9 after HEL priming compared to unprimed mice (Fig. 2A). Because CCR9 is expressed at high levels in the buccal epithelium of unprimed mice (Fig. 2B), this suggests that expression of CCL25 upon antigen exposure may be involved in the development or maturation of T cells locally in the buccal epithelium.

## Discussion

Findings from this study reveal a complex yet logical pattern of chemokine receptor and chemokine ligand gene activation in cells of the buccal epithelium following antigen exposure. The marked increase in the gene expression of CCL19 and its receptor, CCR7, is emblematic of an activation process that involves the local influx of T cells and dendritic cells, especially during the generation of a primary immune response [26]. That pattern is further reflected by the upregulation of CCR5 gene expression, which binds to CCL3 or CCL4 (macrophage inflammatory protein [MIP]-1) and to CCL5 (RANTES [regulated upon activation, normal T cell expressed, and secreted]) [27]. Although we found no change in the gene expression of CCL5, the possibility remains that low level constitutive expression of CCL5 is sufficient to lead to T cell changes through the CCL5/CCR5 pathway.

The upregulated gene expression of CCLl2 (MCP-5) in HEL-primed 3A9 mice was significant due to its ability to bind CCR2 that was constitutively expressed at high levels independent of antigen priming. Because CCR2 participates in T cell/monocyte migration and in the activation of a Th1-type of inflammatory response [21,26], these findings point to a likely process of rapid mononuclear cell mobilization in the buccal epithelium shortly after antigen exposure. Methods for controlling

the regulation in the expression of other important chemokines can be seen in situations in which the use of a specific chemokine is controlled at the level of the chemokine receptor. An example of this in the present study would be the upregulated expression of CCR3, the receptor for CCL11 (eotaxin), the latter being constitutively expressed at high levels, regardless of antigen exposure, thus suggesting that control of leukocyte trafficking, in particular eosinophils [28], into buccal epithelia is controlled at least in part by CCR3 expression. Clearly, however, CCR3 expression also would influence numerous other chemokine-mediated events due to the promiscuous binding of many chemokine ligands (e.g., CCL5, CCL7, CCL8, and others) to CCR3 [26].

One of the most surprising and intriguing findings to emerge from this study was the upregulation of CCL25 (TECK) gene expression in buccal epithelia after HEL priming. Interestingly, the constitutive high level of expression of CCR9, the receptor for CCL25, indicates that the utilization of CCL25 in the buccal epithelium is controlled through the regulated expression of CCL25. Since CCR9 is the only receptor known to be used by CCL25 [29], this further demonstrates the specificity of CCL25 involvement during antigenic stimulation in buccal tissues. Studies from other laboratories describe a very restricted pattern of CCL25 expression that is limited to the thymus and the intestine [24,30–32]. Moreover, even throughout the gastrointestinal tract there is a highly localized expression of CCL25 with highest levels occurring in the duodenum, jejunum, and ileum [24,32] and an absence of expression in the colon [24,31,32] and stomach [24]. Additionally, CCL25 is not expressed in other tissues that have been examined thus far, including respiratory tissues, peripheral lymphoid tissues, and tissues of the central nervous system [24]. The present study is the first to point to a role for CCL25/CCR9 in buccal epithelia and to tie that with an antigen-specific response. The implications of this are notable with regard to the potential for extrathymic T cell development in the oral tissues. In the intestinal mucosa, by way of comparison, CCL25 expression has been localized in villus crypt regions [33], i.e., within areas of the intestinal mucosa believed to be sites of local T cell development and/or maturation. CCL25 also has been linked to the development of TCR$\gamma\delta$ T cells in the thymus [34]. In that vein, recent studies from our laboratory indicate that significant numbers of TCR$\alpha\beta$ and TCR$\gamma\delta$ T cells are present in the buccal epithelium of normal mice ([22]; Wang and Klein, unpublished), and have demonstrated that the V$\gamma$ gene repertoire of buccal $\gamma\delta$ T cells is diverse and closely resembles that of the small intestine [35]. Curiously, there was a dramatic and unexplained transient loss of V$\gamma$ gene expression in buccal epithelia between days 2 and 3 post-antigen exposure by either non-replicating (BSA) or replicating


Fig. 2. (A) Semi-quantitative RT-PCR analyses of CCL25 (TECK) prior to and 24 h after HEL antigen priming. Note the increase in CCL25 gene expression after local HEL antigen exposure of 3A9 mice. (B) CCR9, the receptor for CCL25, is expressed at high levels prior to antigen exposure, and did not change during a three day period studied after antigen priming.

(influenza virus) antigens [22]. Although the basis for that change remains unclear, the findings described here of an active process of CCL25 gene expression following antigen exposure suggest a process in which T cells die or migrate out of epithelial tissues after antigen exposure, and new T cells are then actively recruited into the mucosa. This possibility as a regulatory mechanism for modulating antigen-specific effector cells in the oral mucosa is currently under investigation in our laboratory.

## Acknowledgment

This work was supported by NIH Grant DK35566.

## References

[1] X.Z. Zhou, P.B. Sugerman, N.W. Savage, L.J. Walsh, G. Seymour, Intra-epithelial CD8$^+$ T cells and basement membrane disruption in oral lichen planus, J. Oral Pathol. Med. 31 (2002) 23–27.

[2] M.F. Haque, M. Harris, S. Meghji, A.W. Barrett, Immunolocalization of cytokines and growth factors in oral submucosa bifrosis, Cytokine 10 (1998) 713–719.

[3] A. Pedersen, H.P. Hougen, B. Konrad, T-lymphocyte subsets in oral mucosa of patients with recurrent aphthous ulceration, J. Oral Pathol. Med. 21 (1992) 176–180.

[4] J.R. Klein, R.L. Mosley, Phenotypic and cytotoxic characteristics of IELs, in: H. Kiyono, J.R. McGhee (Eds.), Mucosal Immunology: Intraepithelial Lymphocytes (IELs), Raven Press, New York, 1994, pp. 33–60.

[5] P. Poussier, M. Julius, Thymus independent T cell development and selection in the intestinal epithelium, Annu. Rev. Immunol. 12 (1994) 521–523.

[6] P. Patinen, E. Savilahti, J. Hietanen, M. Malmstrom, M. Maki, T. Reunala, Intraepithelial lymphocytes bearing the $\gamma/\delta$ receptor in the oral and jejunal mucosa in patients with dermatitis herpetiformis, Eur. J. Oral Sci. 105 (1997) 130–135.

[7] I. Bultzingslowen, M. Jontell, Macrophages, dendritic cells and T lymphocytes in rat buccal mucosa and dental pulp following 5-fluorouracil treatment, Eur. J. Oral Sci. 107 (1999) 194–201.

[8] C. Desvignes, F. Esteves, N. Etchart, C. Bella, C. Czerkinsky, D. Kaiserlian, The murine buccal mucosa is an inductive site for priming class I-restricted CD8$^+$ effector T cells in vivo, Clin. Exp. Immunol. 113 (1998) 386–393.

[9] K. Eriksson, E. Ahlfors, A. George-Chandy, D. Kaiserlian, C. Czerkinsky, Antigen presentation in the murine oral epithelium, Immunology 88 (1996) 147–152.
[10] E. Ahlfors, C. Czerkinsky, Contact sensitivity in the murine oral mucosa. I. An experimental model of delayed-type hypersensitivity reactions at mucosal surfaces, Clin. Exp. Immunol. 86 (1991) 449–456.
[11] E. Ahlfors, R. Jonsson, C. Czerkinsky, Experimental T cell-mediated inflammation reactions in the murine oral mucosa. II. Immunohistochemical characterization of resident and infiltrating cells, Clin. Exp. Immunol. 104 (1996) 297–305.
[12] R. Jotwani, A.K. Palucka, M. Al-Quotub, M. Nouri-Shirazi, J. Kim, D. Bell, J. Banchereau, C.W. Cutler, Mature dendritic cells infiltrate the T cell-rich region of oral mucosa in chronic periodontitis: In situ, in vivo, and in vitro studies, J. Immunol. 167 (2001) 4693–4700.
[13] G. Schmalz, H. Schweikl, K.-A. Hiller, Release of prostaglandin $E_2$, IL-6 and IL-8 from human oral epithelial culture models after exposure to compounds of dental materials, Eur. J. Oral Sci. 108 (2000) 442–448.
[14] R. Godiska, D. Chantry, C.J. Raport, S. Sozzani, P. Allavena, D. Leviten, A. Mantovani, P.W. Gray, Human macrophage-derived chemokine (MDC), a novel chemoattractant for monocytes, monocyte-derived dendritic cells, and natural killer cells, J. Exp. Med. 185 (1997) 1595–1604.
[15] C.H. Kim, L.M. Pelus, J.R. White, H.E. Broxmeyer, Differential chemotactic behavior of developing T cells in response to thymic chemokines, Blood 91 (1998) 434–443.
[16] R.M. Strieter, P.J. Polverini, S.L. Kunkel, D.A. Arenberg, M.D. Burdick, J. Kasper, J. Dzuiba, J. Van Damme, A. Walz, D. Marriott, S. Kunkel, The functional role of the ELR motif in CXC chemokine-mediated angiogenesis, J. Biol. Chem. 270 (1995) 27348–27357.
[17] C.S. Tannenbaum, R. Tubbs, D. Armstrong, J.H. Finke, R.M. Bukowski, T.A. Hamilton, The CXC chemokines IP-10 and Mig are necessary for IL-12-mediated regression of the mouse RENCA tumor, J. Immunol. 161 (1998) 927–932.
[18] A.D. Luster, P. Leder, IP-10, a -C-X-C- chemokine, elicits a potent thymus-dependent antitumor response in vivo, J. Exp. Med. 178 (1993) 1057–1065.
[19] E. Nakashima, A. Oya, Y. Kubota, N. Kanada, R. Matsushita, K. Takeda, F. Ichimura, K. Kuno, N. Mukaida, K. Hirose, I. Nakanishi, T. Ujiie, K. Matsuchima, A candidate for cancer therapy: MIP-1 $\alpha$ gene transfer to an adenocarcinoma cell line reduced tumorigenicity and induced protective immunity in immunocompetent mice, Pharm. Res. 13 (1996) 1896–1901.
[20] N.W. Schluger, W.N. Rom, Early responses to infection: chemokines as mediators of inflammation, Curr. Opin. Immunol. 9 (1997) 504–508.
[21] D. Rossi, A. Zlotnik, The biology of chemokines and their receptors, Annu. Rev. Immunol. 18 (2000) 217–242.
[22] K. Otten, H.-C. Wang, P.R. Wyde, J.R. Klein, Modulation of $\gamma\delta$ T cells in mouse buccal epithelium following antigen priming, Biochem. Biophys. Res. Commun. 294 (2002) 626–629.
[23] W.Y. Ho, M.P. Cooke, C.C. Goodnow, M.M. Davis, Resting and anergic B cells are defective in CD28-dependent costimulation of naïve $CD4^+$ T cells, J. Exp. Med. 179 (1994) 1539–1549.
[24] E. Kunkel, J.J. Campbell, G. Haraldsen, J. Pan, J. Boisvert, A.I. Roberts, E.C. Ebert, M.A. Vierra, S.B. Goodman, M.C. Genovese, A.J. Wardlaw, H.B. Greenberg, C.M. Parker, E.C. Butcher, D.P. Andrew, W.W. Agace, Lymphocyte CC chemokine receptor 9 and epithelial thymus-expressed chemokine (TECK) expression distinguish the small intestinal immune compartment: epithelial expression of tissue-specific chemokines as an organizing principle in regional immunology, J. Exp. Med. 192 (2000) 761–767.
[25] S. Uehara, K. Song, J.M. Farber, P.E. Love, Characterization of CCR9 expression and CCL25/thymus-expressed chemokine responsiveness during T cell development: $CD3^{high}$ $CD69^+$ thymocytes and $\gamma\delta TCR^+$ thymocytes preferentially respond to CCL25, J. Immunol. 168 (2002) 134–142.
[26] P.M. Murphy, International union of pharmacology. XXX. Update on chemokine receptor nomenclature, Pharmacol. Rev. 54 (2002) 227–229.
[27] G. Simmons, P.R. Clapham, L. Picard, R.E. Offord, M.M. Rosenkilde, T.W. Schwartz, R. Buser, T.N.C. Wells, A.E. Proudfoot, Potent inhibition of HIV-1 infectivity in macrophages and lymphocytes by novel CCR5 antagonist, Science 276 (1997) 276–279.
[28] M.F. Gurish, A. Humbles, H. Tao, S. Finkelstein, J.A. Boyce, C. Gerard, D.S. Friend, K.F. Austen, CCR3 is required for tissue eosinophilia and larval cytotoxicity after infection with *Trichinella spiralis*, J. Immunol. 168 (2002) 5730–5736.
[29] A. Zaballow, J. Gutierrez, R. Varona, C. Ardavin, G. Marquez, Cutting edge: identification of the orphan chemokine receptor GPR-9-6 as CCR9, the receptor for the chemokine TECK, J. Immunol. 162 (1999) 671–675.
[30] Zabel, W.W. Agace, J.J. Campbell, H.M. Heath, D. Parent, A.I. Roberts, E.C. Ebert, N. Kassam, S. Qin, M. Zovko, G.J. LaRosa, L.-L. Yang, D. Soler, E.C. Butcher, P.D. Ponath, C.M. Parker, D.P. Andrew, Human G protein-coupled receptor GPR-9-6/CC chemokine receptor 9 is selectively expressed on intestinal homing T lymphocytes, mucosal lymphocytes, and thymocytes and is required for thymus-expressed chemokine-mediated chemotaxis, J. Exp. Med. 190 (1999) 1241–1255.
[31] K. Papadakis, J. Prehn, V. Nelson, L. Cheng, S.W. Binder, P.D. Ponath, D.P. Andrew, S.R. Targan, The role of thymus-expressed chemokine and its receptor CCR9 on lymphocytes in the regional specialization of the mucosal immune system, J. Immunol. 165 (2000) 5069–5076.
[32] M.-A. Wurbel, J.-M. Philippe, C. Nguyen, G. Victorero, T. Freeman, P. Wooding, A. Miazek, M.-G. Mattei,, M. Malissen, B.R. Jordan, B. Malissen, A. Carrier, P. Naquet, The chemokine TECK is expressed by thymic and intestinal epithelial cells and attracts double- and single-positive thymocytes expressing the TECK receptor CCR9, Eur. J. Immunol. 30 (2000) 262–271.
[33] N. Onai, M. Kitabatatake, Y.-S. Zhang, H. Ishikawa, S. Ishikawa, K. Matsuchima, Pivotal role or CCL25 (TECK)-CCR9 in the formation of gut cryptopatches and consequent appearance of intestinal intraepithelial T lymphocytes, Int. Immunol. 14 (2002) 687–694.
[34] M.-A. Wurbel, M. Malissen, D. Guy-Grand, E. Meffre, C. Nussenzweig, M. Richelme, A. Carrier, B. Malissen, Mice lacking the CCR9 CC-chemokine receptor show a mild impairment of early T- and B-cell development and a reduction in T-cell receptor $\gamma\delta^+$ gut intraepithelial lymphocytes, Blood 98 (2001) 2626–2632.
[35] M. Whetsell, R.L. Mosley, L. Whetsell, F.V. Schaefer, K.S. Miller, J.R. Klein, Rearrangement and junctional site sequence analyses of T cell receptor gamma genes in intestinal intraepithelial lymphocytes in murine athymic chimeras, Mol. Cell. Biol. 11 (1991) 5902–5909.