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Role of CCL19/21 and its possible signaling through CXCR3 in development of metallophillic macrophages in the mouse thymus

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Abstract

We have already shown that metallophilic macrophages, which represent an important component in the thymus physiology, are lacking in lymphotoxin-β receptor-deficient mice. However, further molecular requirements for the development and correct tissue positioning of these cells are unknown. To this end, we studied a panel of mice deficient in different chemokine ligand or receptor genes. In contrast to normal mice, which have these cells localized in the thymic cortico-medullary zone (CMZ) as a distinct row positioned between the cortex and medulla, in plt/plt

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(paucity of lymph node T cells) mice lacking the functional CCL19/CCL21 chemokines, metallophilic macrophages are not present in the thymic tissue. Interestingly, in contrast to CCL19/21-deficient thymus, metallophilic macrophages are present in CCR7-deficient thymus. However, these cells are not appropriately located in the CMZ, but are mostly crowded in central parts of thymic medulla. The double staining revealed that these metallophillic macrophages are CCR7-negative and CXCR3-positive. In the CXCL13-deficient thymus, the number, morphology and localization of metallophilic macrophages are normal. Thus, our study shows that, CCL19/21and its possible signaling through CXCR3 are required for the development of thymic metallophilic macrophages, whereas the CXCL13-CXCR5 signaling is not necessary.

Keywords

Chemokines; Chemokine receptors; Thymus; Metallophilic Macrophages; Dendritic cells; Mouse

Introduction

The thymus provides a unique inductive microenvironment for the maturation of bone marrow-derived precursor cells into the immunocompetent T-lymphocytes. This microenvironment is composed of various nonlymphoid cells. Several types of epithelial cells and mononuclear phagocytes create the thymic cellular warp and set up the distinct tissue niches suitable for each step of T-lymphocyte maturation to occur (Milićević and Milićević 2004; Miosge and Zamoyska 2007). The molecular mechanisms exerted by thymic nonlymphoid cells, which govern the process of thymocyte maturation, have been studied in greater detail and largely elucidated in recent time (Miosge and Zamoyska 2007). Additionally, it has been shown that the interactions between nonlymphoid cells of the thymic warp and maturing thymocytes are bidirectional: the latter cells control the maturation of respective nonlymphoid cells and induce the formation of microenvironmental niches for their own development (Milićević and Milićević 2004; Gray et al., 2005). However, the molecular mechanisms involved in maturation and maintenance of different thymic nonlymphoid cells are much less understood. Only in very recent years it has been demonstrated that the lymphotoxin-beta (LTβ) signaling pathway represents the crucial regulator of medullary thymic epithelial cell (mTEC) maturation: if LTβ receptor (LTβR) is lacking, the mature subsets of mTEC are absent from the thymic medulla (Milićević et al., 2008; Zhu et al., 2010). This cytokine pathway provides for the secretion of CCL19/21 chemokines (Zhu et al., 2007), which via the respective CCR7 receptor exert the prominent thymocyte attractive activity (Ueno et al., 2004; Kurobe et al., 2006) and the establishment of normal thymic architecture (Förster et al., 2008; Odaka, 2009). The lack of either CCL19/21 or CCR7 interferes with cortex-to-medulla migration of thymocytes and, similarly as the lack of LTβR (Zhu et al., 2007), precludes the negative selection with resulting development of autoimmune manifestations (Kurobe et al., 2006; Nitta et al., 2009).

Metallophilic macrophages are members of thymic mononuclear phagocyte cell population. In contrast to cortical macrophages and medullary dendritic/interdigitating cells, metallophilic macrophages are located in the thymic cortico-medullary zone (CMZ), that is,

at the junction of cortex and medulla, and show a number of special morphological and functional features (Milićević and Milićević 2004). These cells are clearly related to thymocytopoiesis (Milićević et al., 1983) and very likely to participate in the process of thymic negative selection. The latter view is supported by the fact that thymic metallophilic macrophages: (i) show the oversized antigen-presenting cellular machinery (Milićević et al., 1987; Milićević and Milićević 2000), (ii) are strategically positioned in the CMZ (Milićević and Milićević 2004), which is generally believed to be the place where clonal deletion normally occurs in the thymus (Hogquist et al. 2005; Sprent and Kishimoto 2002), (iii) undergo dramatic changes (Milićević et al. 1989; Milićević and Milićević 1998) after inhibition of negative selection with cyclosporine A (Jenkins et al. 1988), and (iv) are lacking in lymphotoxin-β receptor (LTβR)-deficient mice (Milićević et al. 2006), which are prone to development of autoimmunity (Zhu et al., 2007).

Recently, we have shown that, in contrast to LTβR-deficient mice and animals with disrupted LTβ/LTβR signaling pathway (Milićević et al., 2006), the presence of AutoImmune Regulator (AIRE) transcription regulator is not necessary for the development of thymic metallophilic macrophages (Milićević et al., 2009). However, nothing else is presently known about the molecular mechanisms involved in maturation and development of thymic metallophilic macrophages. Considering that the expression of chemokines and their receptors is of the utmost significance for the development of mature mTEC populations and successful negative selection (Worbs and Förster, 2007) and that development of metallophilic macrophages is finely regulated in coordination with mTEC development (Milićević et al., 2006; Milićević et al., 2008), we believed that both types of thymic nonlymphoid cells could be subject to same molecular regulatory mechanisms. Therefore, our aim was to investigate the presence of metallophilic macrophages in the thymus of mice deficient in various chemokines and their receptors.

Materials and methods

Animals

Normal C57BL/6 mice were purchased from Charles River Laboratories. Originally, CCL19/21-deficient (paucity of lymph node T cells: plt/plt) (Nakano et al., 1997) and CXCL13-deficient (Ansel et al., 2000) mice were obtained from Dr. J.G. Cyster (University of California, San Francisco, CA). Double knock-out (CCL19/21)-CXCL13-deficient mice were generated at the Trudeau Institute, Saranac Lake, NY. All gene-targeted mice were on the C57BL/6 genetic background and were bred at the animal breeding facility of the Department of Medicine, University of Rochester Medical Center, NY. All procedures using these animals were approved by the University of Rochester University Committee on Animal Resources and were conducted according to the principles outlined by the National Research Council. CCR7-deficient mice (Förster et al., 1999) were bred and maintained at the mouse facility of the Institute of Molecular and Cell Biology, Tartu University. The permission to perform these animal experiments was issued by the Estonian State Committee for Licensing of Animal Experiments.

Tissue preparation, silver impregnation, enzymehistochemistry and immunohistochemistry

At present, the antibodies specific for mouse thymic metallophilic macrophages do not exist. Monoclonal antibody MOMA-1 is successfully used for demonstration of metallophilic macrophages in the mouse spleen (Kraal and Janse 1986). But, it is not functional in the mouse thymus and the use of silver impregnation cannot be avoided for detection of thymic metallophilic macrophages. Furthermore, as these cells are rich in lysosomes (Milićević and Milićević 2004), the lysosomal markers, for example, acid phosphatase (AcP; Leder and Stutte, 1975) and Lysosome-Associated Membrane Protein 1 (LAMP-1; Chen et al. 1985) may be also used for detection of thymic metallophilic macrophages in addition to silver impregnation. In such preparations metallophilic macrophages become evident due to their voluminous appearance, stronger AcP or LAMP-1 reactivity and characteristic positioning in the CMZ – these features enable their unambiguous identification.

Thymuses from 8–10 weeks old mice of both sexes were used. Pieces of thymic tissue, fixed in neutral buffered formaldehyde or Bouin's solution, were routinely processed for paraffin wax sectioning. For cryostat sectioning, thymic tissue quickly frozen in liquid nitrogen was used. Paraffin sections, 3–5 μm thick, were stained with hematoxylin-eosin or impregnated with ammoniacal silver according to Weil-Davenport method (Bancroft and Stevens 1982). Cryostat sections of unfixed thymic tissue, 5–7 μm thick, were used for enzymehistochemical detection of AcP (Leder and Stutte, 1975) and for immunohistochemical demonstration of LAMP-1 (Chen et al. 1985). The purified rat anti-mouse CD107a (clone 1D4B) was used (BD Biosciences, Heidelberg, Germany). In brief, cryostat sections were air-dried at room temperature for 2 hours and fixed in a 1:1 (v/v) mixture of methanol-acetone for 10 minutes at −20°C followed by 4 % paraformaldehyde for 45 minutes at 4°C. Sections were incubated with primary antibody for 1 hour and staining was revealed by incubation with rabbit-anti-rat-Ig and RAPAAP each for 30 minutes followed by FastBlue BB (Sigma Aldrich, Steinheim, Germany). Alternatively, cryostat sections were air-dried at room temperature, fixed with 4% paraformaldehyde, permeabilized with 0.3 % Triton®X-100 (Applichem GmbH, Darmstadt, Germany) and blocked with normal goat serum (DakoCytomation, Glostrup, Denmark). Sections were incubated with 1:200 of primary antibody, rat monoclonal anti-mouse CD107a at 4°C overnight, followed by 2 hour incubation at room temperature with 1:2000 of goat anti-rat IgG AlexaFluor® 488 (Molecular Probes, Eugene, OR, USA). The sections were counterstained with DAPI (Roche Diagnostics GmbH, Mannheim, Germany). The double staining with LAMP-1 and CCR7 or CXCR3 was performed as follows. In the first case, the rabbit monoclonal primary antibody (CCR7 antibody; ab32527) diluted at 1:500 and goat polyclonal secondary anti-rabbit IgG Chromeo™ 488 (ab60314) diluted at 1:2,000 (Abcam, Cambridge, UK) were used. In the second case, rabbit polyclonal primary antibody (CD183/CXCR3 antibody; 251386; Abbiotec, San Diego, CA, USA) diluted at 1:100 at 4°C overnight, followed by secondary goat anti-rabbit IgG AlexaFluor® 488 (Molecular Probes) diluted at 1:2,000 for 1 h at room temperature, were used. The images were acquired by fluorescence microscopy (Eclipse TE2000-4; Nikon, Melville, NY, USA).

Results

Normal thymus

The thymus of normal C57BL/6 mice shows a well-developed cortex and medulla, with a clear boundary between these regions. Metallophilic macrophages are predominantly localized in the CMZ, as a distinct row positioned between the cortex and medulla, neatly apposed to the innermost layer of cortical thymocytes (Fig. 1a). Some rare cells are scattered throughout the medulla, whereas metallophilic macrophages are absent from the thymic cortex. Metallophilic macrophages are large cells, with abundant cytoplasm and very prominent, stocky cytoplasmic prolongations (Fig. 1b). Similarly to cortical macrophages, metallophilic macrophages of the CMZ are AcP-positive. But, they can easily be distinguished from cortical macrophages due to their strict position in the CMZ, larger size and more intense AcP reaction (Fig. 1c). Some interdigitating cells with characteristic spot-like AcP-positive reaction are seen in the thymic medulla. Due to the abundance of specialized endocytic compartments, metallophilic macrophages show up in anti-LAMP-1 immunostained sections of thymic tissue. These cells are very large, show stronger LAMP-1-positive reaction and, thus, can be easily distinguished from cortical macrophages and medullary interdigitating cells (Fig. 1d), the latter showing a characteristic spot-like positive reaction.

CCL19/21-deficient thymus

The thymus of CCL19/21-deficient mice has a well-developed cortex, densely packed with thymocytes. The thymic medulla is, however, markedly diminished in size, mostly reduced to smaller, isolated areas and less well populated with thymocytes. Nevertheless, the boundary between the cortex and medulla is clearly discernible. Large, dilated blood vessels are more frequently seen than in the normal thymus. Silver impregnation shows that metallophilic macrophages are absent from the CMZ of CCL19/21-deficient thymus (Fig. 1e; Table 1). Rare metallophilic cells are seen in the inner and central parts of the residual medullary islands. But, these cells are morphologically different from normal CMZ metallophilic macrophages: they are much smaller, rounded in shape, with scanty cytoplasm and very inconspicuous prolongations (Fig. 1f). The absence of metallophilic macrophages from the CMZ of CCL19/21-deficient thymus is further confirmed by the AcP staining. Although the macrophages are noticeable in the cortex of CCL19/21-deficient thymus, the rim of metallophilic, strongly AcP-positive macrophages in the CMZ is completely lacking (Fig. 1g). This finding is further corroborated by the absence of large, LAMP-1-positive, metallophilic macrophages from thymic CMZ (Fig. 1h).

CCR7-deficient thymus

Grossly, the thymus of CCR7-deficient mice is very similar to that of CCL19/21-dificient animals with well-developed thymic cortex and medulla reduced in size. However, very surprisingly, in contrast to CCL19/21-deficient thymus, large metallophilic cells are scattered throughout the thymic medulla of CCR7-deficient thymus (Fig. 2a; Table 1). These cells also show the strong LAMP-1 reaction (Fig. 2b). On the other hand, only rare large metallophilic macrophages are observed in the CMZ of CCR7-deficient thymus (Fig. 2a). Notable is the presence of numerous, dilated blood vessels (not shown). This finding

suggested that CCR7 is irrelevant for the appropriate development of thymic metallophilic macrophages. To test this view, we performed the double staining of normal thymus sections with LAMP-1 and CCR7. We revealed that, indeed, the large LAMP-1-positive metallophilic macrophages are CCR7-negative (Fig. 3). As it has been shown that CCL21 may also act as ligand for CXCR3 receptor (Soto et al. 1998), we next performed the double staining of normal and CCR7-deficient thymus sections with LAMP-1 and CXCR3. We revealed that, in addition to other double-positive cells, large LAMP-1-positive metallophilic macrophages also show the clear CXCR3-positive reaction (Fig. 4 a–c). Notably, the staining for CXCR3 in the cortex is very weak, whereas in the medulla numerous CXCR3-positive cells are seen, some of which showing the characteristic spot-like LAMP-1-positive reaction may correspond to plasmacytoid dendritic cells (Penna et al. 2002).

(CCL19/21)-CXCL13-double-deficient thymus

Structurally, the thymus of (CCL19/21)-CXCL13-double-deficient mice is very similar to that of CCL19/21-dificient animals: the thymic cortex is well preserved, whereas the medulla is reduced to small areas much less populated with thymocytes. The presence of dilated blood vessels is very notable. Metallophilic macrophages are absent from the thymic CMZ and only very scanty metallophilic cells of atrophic appearance may be seen in the inner regions of the medulla (Fig. 5a; Table 1). These findings are confirmed by AcP and LAMP-1 staining (not shown).

CXCL13-deficient thymus

General morphologic features of the CXCL13-deficient thymus are very similar to those of the normal thymus, with well-developed cortex and medulla. The position of metallophilic macrophages in the CMZ, as well as their number and appearance, largely correspond to that of the normal thymus (Fig. 5b; Table 1). The AcP and LAMP-1 staining also confirm these findings (not shown).

Discussion

We have already shown that thymic metallophilic macrophages are lacking in LTβR-deficient mice (Milićević et al., 2006). This finding is in good keeping with the results obtained in the present work, which documents the derangement of these cells in the thymus of mice lacking CCL19/21. LTβR pathway has been shown to play the role of a master regulator in development and structural organization of secondary lymphatic organs (Fütterer et al., 1998; van de Pavert and Mebius 2010). Ligation of LTβR induces the alternative signaling pathway of nuclear factor kappaB activation (Dejardin 2006) with subsequent regulation of target genes and production of tissue-organizing chemokines CXCL13 and CCL19/21 by stromal cells, which induce the segregation of B- and T-lymphocytes respectively, via the corresponding CXCR5 and CCR7 receptors (Schneider et al., 2004; Ngo et al., 1999).

In this study, we clearly show that CCL19/21 is required for the development and correct positioning of thymic metallophilic macrophages in the CMZ—immunohistochemical

staining demonstrated that CCL21 expression is lacking in plt/plt thymus (Misslitz et al. 2004). Surprisingly, the metallophilic macrophages are not absent from CCR7-deficient thymus, which stands in contrast to CCL19/21-deficient thymus. Initially, we hypothesized that CCL19/21 signaling regulates the development of thymic metallophilic macrophages through CCR7 either directly or indirectly via thymocytes, which are known to express this chemokine receptor—CCL19/21 chemokines produced by medullary epithelium induce the migration of positively selected thymocytes to the medulla where they influence the development of medullary epithelial cells and potentially also metallophilic macrophages (Förster et al. 2008; Kurobe et al. 2006; Nitta et al. 2009). However, the presence of metallophilic macrophages in the medulla of CCR7-deficient thymus shows that this receptor is not instrumental for the CCL19/21-controlled development of these cells. The explanation for this finding could be that, in the absence of CCR7, some other receptor is operative in development of metallophilic macrophages induced by CCL19/21 signaling. A very good candidate is CXCR3 chemokine receptor. It has been shown that CCL21 may act as ligand for CXCR3, as an example of a CC chemokine that specifically binds a CXC receptor (Soto et al. 1998). Moreover, after injury the damaged neurons express CCL21 which activates CXCR3 to trigger the chemotaxis of murine microglia (Rappert et al. 2002). Indeed, using the double immunohistochemical staining with LAMP-1 and CCR7 or CXCR3 we demonstrated that metallophilic macrophages in the mouse thymus are CCR7-negative and CCR3-positive. In accordance with these facts, it seems possible that the preserved CCL21 expression in CCR7-deficient thymus (Witt and Robey 2004) may induce the development of metallophilic macrophages via CXCR3 signaling.

However, although present within the CCR7-deficient thymus, metallophilic macrophages are not appropriately located in the CMZ, but mostly crowded in central parts of thymic medulla. The incorrect positioning of these cells possibly reflects the accumulation of $CD25^{+}CD44^{+}$ subset of early thymocyte progenitors in the CMZ of CCR7-deficient thymus which may dislocate the metallophilic macrophages toward medulla (Misslitz et al. 2004).

Taken together with data from the literature, our results illustrate the complementary control exerted by T- and B cell chemoattractive cytokines (CCL19/21 and CXCL13, respectively), which not only navigate the respective lymphocyte populations toward the correct positions in the lymphoid tissues (Ngo et al. 1999; Schneider et al. 2004), but also regulate the development and positioning of functionally related metallophilic macrophage populations in the thymus (this work) and spleen (Ato et al. 2004; Poljak et al. 1999; Table 1). In this manner, the chemokines bring together the lymphocytes and corresponding metallophilic macrophages to the appropriate tissue location suitable for their cooperation. It was shown that LTβR signaling and subsequent CXCL13–CXCR5 activation are required for the development of marginal metallophilic macrophages in the spleen (Matsumoto et al. 1997; Poljak et al. 1999). Experimental disruption of these signaling pathways induces the loss of metallophilic macrophages from the splenic marginal sinus (Poljak et al. 1999), whereas in plt/plt mice lacking the functional CCL19/CCL21 chemokines the splenic marginal metallophilic macrophages remain undisturbed (Ato et al. 2004). Thus, CXCL13 guides the B cells towards the functionally vital location (Ansel et al. 2000) and at the same time controls the development of splenic marginal metallophilic macrophages (Poljak et al. 1999), which transport the antigens into the lymphoid follicles (Martínez-Pomares and

Gordon 1999). On the other hand, CCL19/21 directs the maturing thymocytes into the thymic medulla (Ueno et al. 2004), but also controls the development/positioning of metallophilic macrophages (this work), which may be vital for the process of negative selection occurring during this journey (Kurobe et al. 2006; Nitta et al. 2009). The different molecular requirements for the development of metallophilic macrophages in the thymus and spleen (summarized in Table 1) highlight the functional differences between these cell types.

In conclusion, we show that CCL19/21 and its possible signaling through CXCR3 are required for the development of thymic metallophilic macrophages, whereas the CXCL13–CXCR5 signaling is not necessary.

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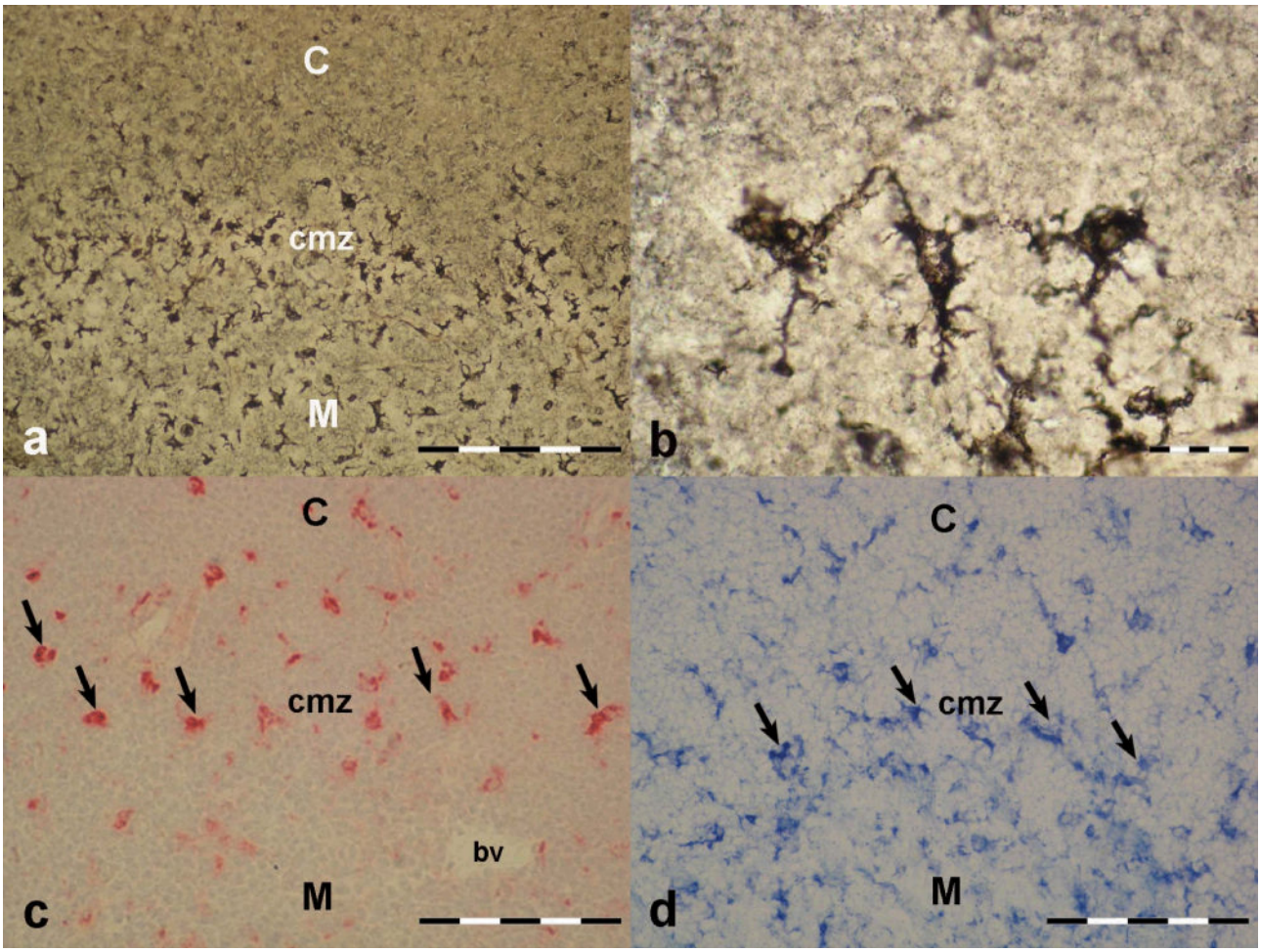

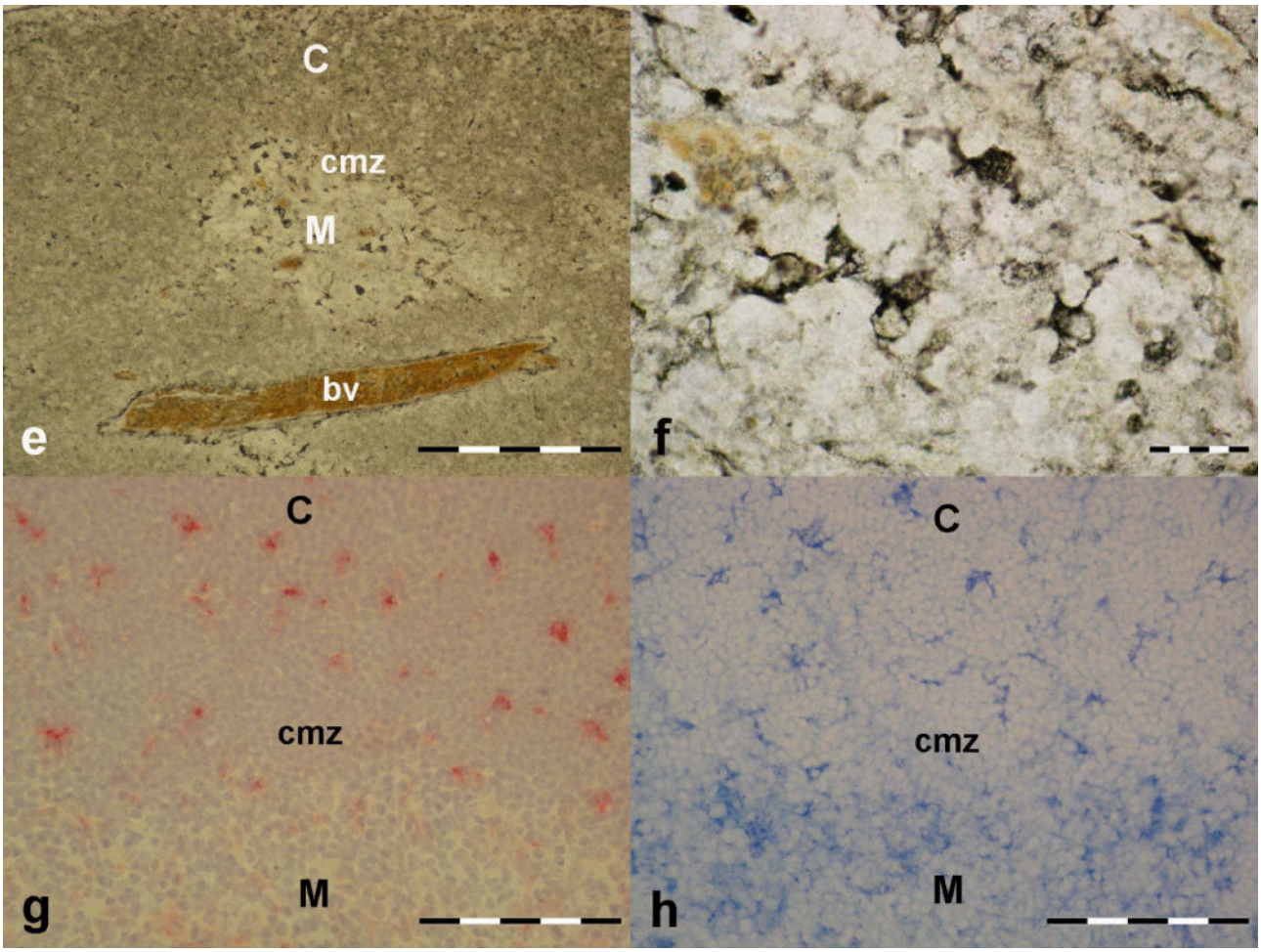

Fig 1.

Normal thymus (**a**–**d**) and CCL19/21-deficient thymus (**e**–**h**). **a** In the normal mouse thymus metallophilic macrophages are strategically positioned in the cortico-medullary zone (cmz), between the cortex (C) and medulla (M). **b** Metallophilic macrophages of the normal mouse thymus are large cells with abundant cytoplasm and prominent, stocky cytoplasmic prolongations. **c** Acid phosphatase-positive metallophilic macrophages (some indicated by *arrowheads*) are positioned in the cortico-medullary zone (cmz). They are larger and show a more intense acid phosphatase reaction than macrophages in the thymic cortex (C) and interdigitating cells in the medulla (M). **d** Large LAMP-1-positive metallophilic macrophages (some indicated by *arrowheads*) are prominent in the cortico-medullary zone (cmz) of the normal mouse thymus. These cells are larger and show a stronger reaction than the cells in the cortex (C) and medulla (M). **e** Metallophilic macrophages are lacking in the cortico-medullary zone (cmz) of the CCL19/21-deficient thymus. Thymic medulla (M) is reduced in size and numerous dilated blood vessels (bv) are seen. **f** Only some rare metallophilic cells present in the residual medullary islands of the CCL19/21-deficient thymus. But, these cells are much smaller, with scanty cytoplasm and rounded in shape, with frail prolongations **g** The large, acid phosphatase-positive metallophilic macrophages are lacking in the cortico-medullary zone (cmz) of the CCL19/21-deficient thymus, although

numerous acid phosphatase-positive macrophages are present in the cortex (C). **h** LAMP-1-positive metallophilic macrophages are lacking in the cortico-medullary zone (cmz) of the CCL19/21-deficient thymus. LAMP-1-positive macrophages are preserved in the thymic cortex (C). C=cortex; M=medulla. a, e, bar=200 μm; c, d, g, h, bar=100 μm; b, f, bar=20 μm

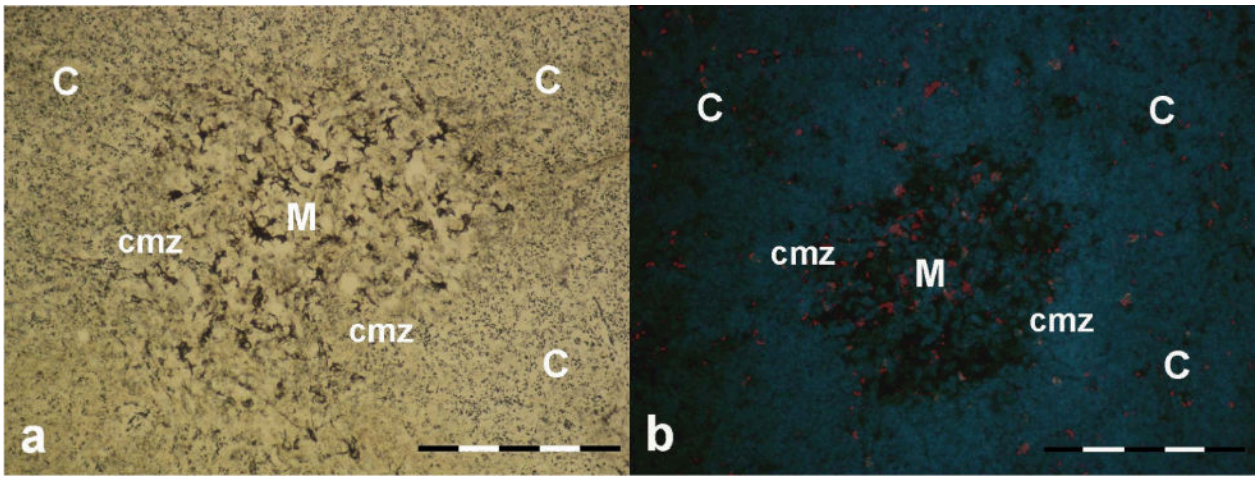

Fig. 2.

CCR7-deficient thymus. **a** Large metallophilic macrophages are crowded in the center of the thymic medulla (M) and only rarely positioned in the cortico-medullary zone (cmz). **b** Large LAMP-1-positive metallophilic macrophages are present in central part of the thymic medulla (M) and not in the cortico-medullary zone (cmz). C=cortex. Bar=200 μm

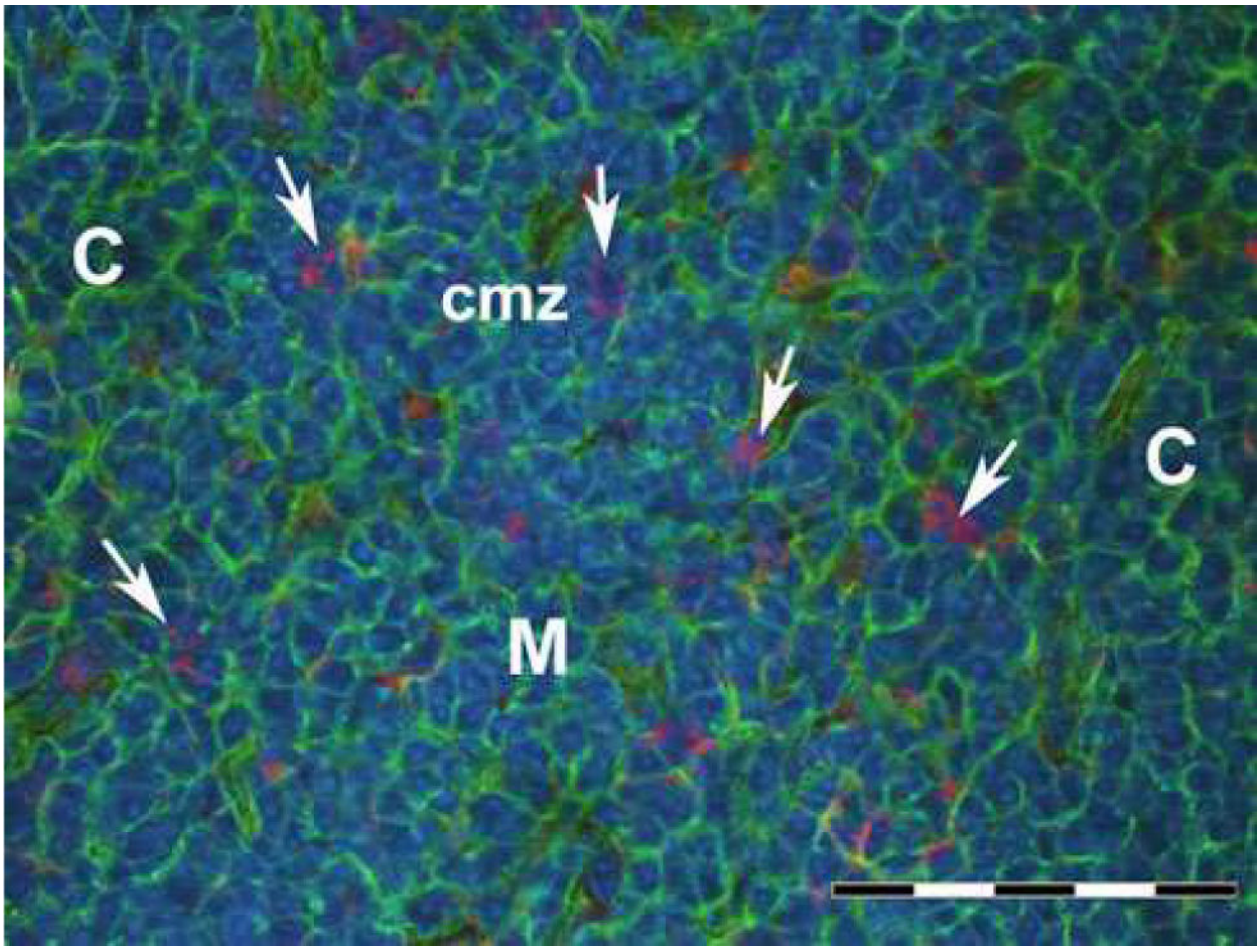

Fig. 3.

Normal thymus, double staining with LAMP-1 (red) and CCR7 (green). Large LAMP-1-positive metallophilic macrophages (some indicated by arrows) positioned in the cortico-medullary zone (cmz) do not show the positive staining with CCR7. Counterstained with DAPI. C cortex; M medulla. Bar 100 μm

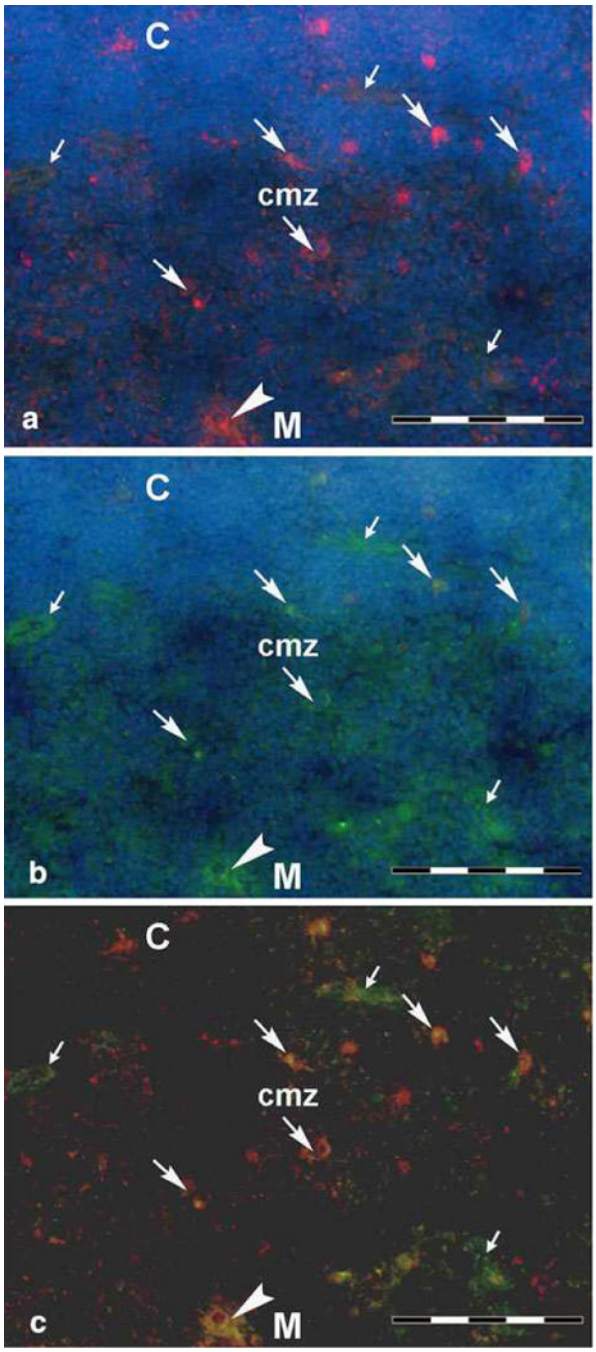

Fig. 4.

Normal thymus, double staining with LAMP-1 (a), CXCR3 (b) and merge (c). Thymic cortex (**C**) is densely packed with thymocytes, whereas in the medulla (**M**) they are loosely arranged. Large metallophilic macrophages (some indicated by **arrows**) positioned in the cortico-medullary zone (**cmz**) show the intense LAMP-1-positive (a) and CXCR3-positive (b) reaction, the yellow fluorescence indicates the double-positive staining (c). Counterstained with DAPI. **Arrowhead** Hassall's corpuscle in the medulla; **small arrows** CXCR3-positive perivascular fibroblasts. **Bar** 100 μm

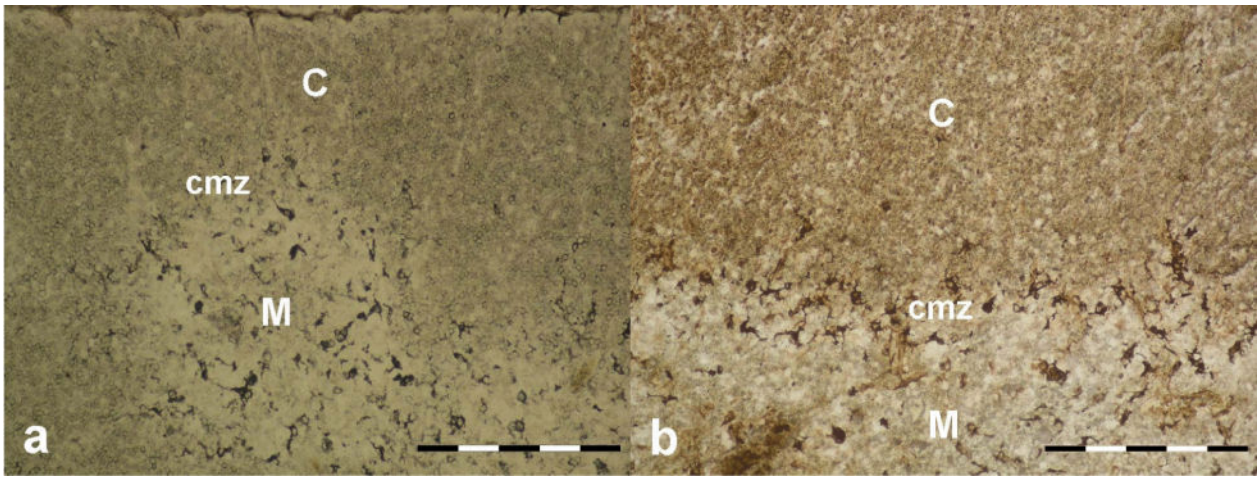

Fig. 5.

(CCL19/21)-CXCL13-double-deficient thymus **(a)** and CXCL13-deficient thymus **(b)**. **a** Metallophilic macrophages are absent from the cortico-medullary zone (cmz) and only very few cells of atrophic appearance are present in the medulla. **b** The number and appearance of metallophilic macrophages in the cortico-medullary zone (cmz) closely correspond to those of normal animals. C=cortex; M=medulla. Bar=200 μm

Table 1

Presence of metallophilic macrophages in the thymic cortico-medullary zone (CMZ) and splenic marginal zone (MZ) of various mutant mouse strains

Molecule	Thymic CMZ	Splenic MZ
LTβR$^{-/-}$	− (Milićević et al., 2006)	− (Fütterer et al., 1998)
NIK$^{-/-}$	− Milićević et al., 2006)	− (Koike et al., 1996)
CCL19/21$^{-/-}$	− (this work)	+ (Ato et al., 2004)
(CCL19/21)-CXCL13$^{-/-}$	− (this work)	NA
(p52/NF-kappaB$^{-/-}$) CXCL13$^{-/-}$	+ (this work)	− (Poljak et al., 1999)
CCR7$^{-/-}$	+ (this work)[a]	NA
TNFR-1$^{-/-}$	+ (Milićević et al., 2006)	+ (Matsumoto et al., 1997)
Aire$^{-/-}$	+ (Milićević et al., 2009)	+ (Hässler et al., 2006)

LTβR$^{-/-}$ = Lymphotoxin-β receptor-deficient; NIK$^{-/-}$ = Nuclear factor-kappaB-inducing kinase-deficient; CCL19/21$^{-/-}$ = CCL19/21 chemokine ligand-deficient; (CCL19/21)-CXCL13$^{-/-}$ = (CCL19/21)-CXCL13 chemokine ligand-double-deficient; (p52/NF-kappaB$^{-/-}$) CXCL13$^{-/-}$ = (p52 subunit of NF-kappaB) CXCL13 chemokine ligand-deficient; CCR7$^{-/-}$ = CCR7 chemokine receptor-deficient; TNFR-1$^{-/-}$ = Tumor necrosis factor receptor-1-deficient; Aire$^{-/-}$ = Autoimmune regulator-deficient; NA = Data not available;

[a] Incorrect positioning