The role of the atypical chemokine receptor CCX-CKR in thymocyte development and its influence on the link between innate and adaptive immunity through regulation of dendritic cell migration

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Declaration

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Abbreviations

Ab	Antibody
APC	Antigen presenting cell
BM	Bone marrow
BMDC	Bone marrow-derived dendritic cell
BrdU	5-Bromo-2'-Deoxyuridine
BSA	Bovine serum albumin
CCL	CC chemokine ligand
CFA	Complete Freund's adjuvant
CFSE	Carboxyfluorescein diacetate succinimidyl ester
CHS	Contact hypersensitivity
CMJ	Cortico-medullary junction
cTEC	Cortical thymic epithelial cell
CXC	CXC chemokine ligand
DC	Dendritic cell
dDC	Dermal dendritic cell ($CD207^+CD11b^+$, $CD207^-CD11b^{hi}$, $CD207^-CD11b^-$)
DEPC	Diethyl pyrocarbonate
DMSO	Dimethyl sulfoxide
DN	Double negative ($CD4^-CD8^-$)
DP	Double positive ($CD4^+CD8^+$)
ELISA	Enzyme-linked immunosorbent assay
FCS	Foetal calf serum
FITC	Fluorescein isothiocyanate
Flt3L	FMS-like tyrosine kinase 3 ligand
FMO	Fluorescence minus one
FRC	Fibrobalstic reticular cell
GAG	Glycosaminoglycan
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GPCR	G-protein coupled receptor
H&E	Haematoxylin and eosin
HBSS	Hank's balanced salt solution
HEV	High endothelial venule
HRP	Horseradish peroxidise
IF	Immunofluorescence
IL	Interleukin
IV	Intravenous
LC	Langerhans cell ($CD207^+CD11b^+$)
Lin	Lineage

LPS	Lipopolysaccharide
LTi cell	Lymphoid tissue inducer cell
MHC	Major histocompatability complex
mTEC	Medullary thymic epithelial cell
NGS	Normal goat serum
NMS	Normal mouse serum
NRS	Normal rat serum
nTreg	Natural regulatory T cell
PBS	Phosphate buffered saline
pDC	Plasmacytoid dendritic cell
PCR	Polymerase chain reaction
RTE	Recent thymic emigrant
SCS	Sub-capsular sinus
SCZ	Sub-capsular zone
SLO	Secondary lymphoid organs
SP	Single positive ($CD4^+CD8^-$ or $CD8^+CD4^-$)
TBS	Tris buffered saline
TCR	T cell receptor
TCZ	T cell zone
TEC	Thymic epithelial cell
TNF	Tumour necrosis factor

Publications arising from this work

Manuscripts

Bunting MD, Comerford I, McColl SR. Finding their niche: chemokines directing cell migration in the thymus. Immunol Cell Biol., 2011 Feb;89(2):185-96

Bunting MD, Comerford I, Seach N, Hammett MV, Asquith DL, Korner H, Boyd RL, Nibbs RJB, McColl SR. CCX-CKR regulates CCL25 distribution within the thymus, promotes outward migration of DN thymocytes and reduces spontaneous autoimmunity. Submitted - Blood.(June, 2012)

Bunting MD, Comerford I, McColl SR. CCX-CKR controls peripheral dendritic cell migration by regulating CCL21 in the skin. Manuscript in preparation

Comerford I, Nibbs RJ, Litchfield W, **Bunting MD**, Harata-Lee Y, Haylock-Jacobs S, Forrow S, Korner H, McColl SR. The atypical chemokine receptor CCX-CKR scavenges homeostatic chemokines in circulation and tissues and suppresses Th17 responses. Blood, 2010, Nov 18;116(20):4130-40

Conference proceedings

Australian Society for Immunology (SA / NT Branch) 4th Adelaide Immunology Retreat (2008): Oral Presentation entitled 'Investigation of the role of CCX-CKR in dendritic cell function'

The Australian Society for Medical Research (ASMR) South Australian Meeting (2009): Poster entitled 'Role of the atypical chemokine receptor CCX-CKR in dendritic cell function'

Gordon Research Conference: Chemotactic Cytokines in Lucca, Italy (2010): Poster entitled 'The atypical chemokine receptor CCX-CKR regulates thymocyte development'

Australian Society for Immunology (SA / NT Branch) 6th Adelaide Immunology Retreat (2010): Oral presentation entitled 'Role of the atypical chemokine receptor in thymic function'

Australian Society for Immunology (SA / NT Branch) 7th Adelaide Immunology Retreat (2011): Oral presentation entitled ‘Deletion of the atypical chemokine receptor CCK-CKR disrupts the thymic CCL25 gradient, alters thymocyte development and leads to spontaneous Sjögren’s-like autoimmunity’ (Won best PhD presentation)

Australasian Society for Immunology International Conference (2011): Oral presentation entitled ‘CCX-CKR: A novel regulator of thymic chemokines, thymocyte development and self-tolerance’

Abstract

The significance of chemokines in directing cell migration both during homeostasis and immune responses has been appreciated for some time. However, the mechanisms in place to post-translationally regulate cell migration through chemokine modulation are only recently becoming clear. CCX-CKR is a receptor that can scavenge and degrade the ligands of CCR7 and CCR9, two receptors that are crucial during instruction of T cell development in the thymus and dendritic cell migration for initiation of adaptive immune responses.

Within the thymus CCL19, CCL21 and CCL25 direct CCR7- and CCR9-expressing thymocytes through distinct thymic compartments, enabling development of a self-MHC restricted and self-tolerant peripheral T cell repertoire. Yet mechanisms outside of transcriptional control that are involved in thymic chemokine regulation have not been well characterised. The aim of this study was to thoroughly investigate the role of CCX-CKR expression on chemokine regulation in the thymus and thymocyte development. Expression of CCX-CKR was detected primarily in cortical thymic epithelial cells, with modest contributions from other thymic stromal populations. Deletion of CCX-CKR led to thymic architecture alterations, reduced levels of CCL19 and CCL25 and a profound decrease in CCL25 (protein) within the cortex. These alterations in chemokine levels and distribution were associated with several defects in the frequency and localisation of thymocyte precursors. Specifically, in CCX-CKR$^{-/-}$ thymi, precursor double-negative 2 (DN2) cells accumulated in the medulla and reduced frequencies of DN3 cells were apparent, coincident with reduced numbers of DN3 cells in the cortex. These observations are likely to be the combined outcome of impaired expansion of cortical thymic epithelial cells and reduced outward migration signals in CCX-CKR$^{-/-}$ thymi. Additionally, CCX-CKR$^{-/-}$ thymi contain increased numbers of CD4^{+}CD8^{+} double-positive, CD4^{+} single-positive and CD8^{+} single-positive cells. Together, these thymic defects were associated with enhanced incidence of inflammatory pathology resembling Sjögren's syndrome, characterised by lymphocytic infiltrates in salivary glands and liver of 8-10 month old CCX-CKR$^{-/-}$ mice.

Previous work has implicated CCX-CKR as an important regulator of CCL19 and CCL21 *in vivo* and deletion of CCX-CKR led to early onset of experimental autoimmune encephalomyelitis (EAE), a T cell mediated autoimmune disease. CCX-CKR was also implicated in promoting effective induction of adaptive immune responses in the LN as

evidenced by abrogated T cell proliferation. An important component of both peripheral tolerance induction and initiation of adaptive immune responses is CCR7-dependent migration of antigen-loaded peripheral dendritic cells and naïve T cells to secondary lymphoid organs where antigen presentation to T cells occurs. The contribution of CCX-CKR to these processes was investigated in CCX-CKR$^{-/-}$ mice. Short-term homing of CD4^{+} T cells to the lymph nodes of CCX-CKR$^{-/-}$ mice was impaired yet homeostatic maintenance of T and B cells remained undisturbed. CD207^{+} skin-derived DCs were significantly less abundant in the lymph nodes of CCX-CKR$^{-/-}$ mice during both steady-state and inflammation which was associated with reduced numbers of CD207^{+} dermal dendritic cells and increased levels of CCL21 in the skin. Furthermore, during CFA-induced inflammation, both migratory and lymph node-resident dendritic cell numbers were abrogated in the lymph nodes, but not spleen, of CCX-CKR$^{-/-}$ mice.

Together, these data identify a novel role for CCX-CKR in maintenance of the thymic cortical compartment that is associated with effective thymocyte development. Moreover, CCX-CKR is required to maintain the homeostatic and inflammatory migration of tolerogenic and activating dendritic cells, respectively, to the lymph nodes. Thus, the combination of thymic and skin/LN associated CCX-CKR establishes optimal conditions for central and peripheral tolerance induction leading to the development of self-tolerant adaptive immune responses.