Review Article

Chemokines in inflammatory and immune diseases

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Nearly two decades have passed since our discovery of the prototypic chemokines, a neutrophil chemotactic factor interleukin 8 (IL 8, CXCL8) and monocyte chemotactic factor MCAF/MCP-1 (CCL2) at the National Cancer Institute, USA. The characterization of chemokines has revealed the molecular mechanisms underlying specific leukocyte subset infiltration into inflammatory tissues, previously a long-standing enigma in inflammation research. In this review we briefly recount the chronology of chemokine research, then overview chemokine receptor signal transduction systems, chemokines in inflammation and immunity, chemokines in HIV infection and cancer, and the current status of therapeutic approaches targeting chemokines and their receptors.

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Introduction

Inflammatory and immune responses are evoked as a host defense against various environmental stimuli. These include external stresses such as infection by pathogens, inhalation of foreign materials like asbestos, exposure to heavy metals, ultraviolet and ionizing radiation as well as internal stresses such as excessive accumulation of metabolites like urate crystals and oxidized lipids, superoxide and nitric oxide, autoimmune responses and cancer. Excessive and persistent inflammation causes various human diseases and also regulates the pathophysiological state of existing diseases. Recent advancements in understanding innate recognition systems and the molecular bases of leukocyte dynamics indicate that inflammation and antigen-specific immune responses are tightly connected (Fig. 1).

The infiltration of specific leukocyte subset into the tissues in inflammatory and immune diseases is inevitable, although the molecular mechanisms underlying leukocyte infiltration for a long time remained an enigma. The discovery of chemokines and leukocytes adhesion molecules has solved this mystery.

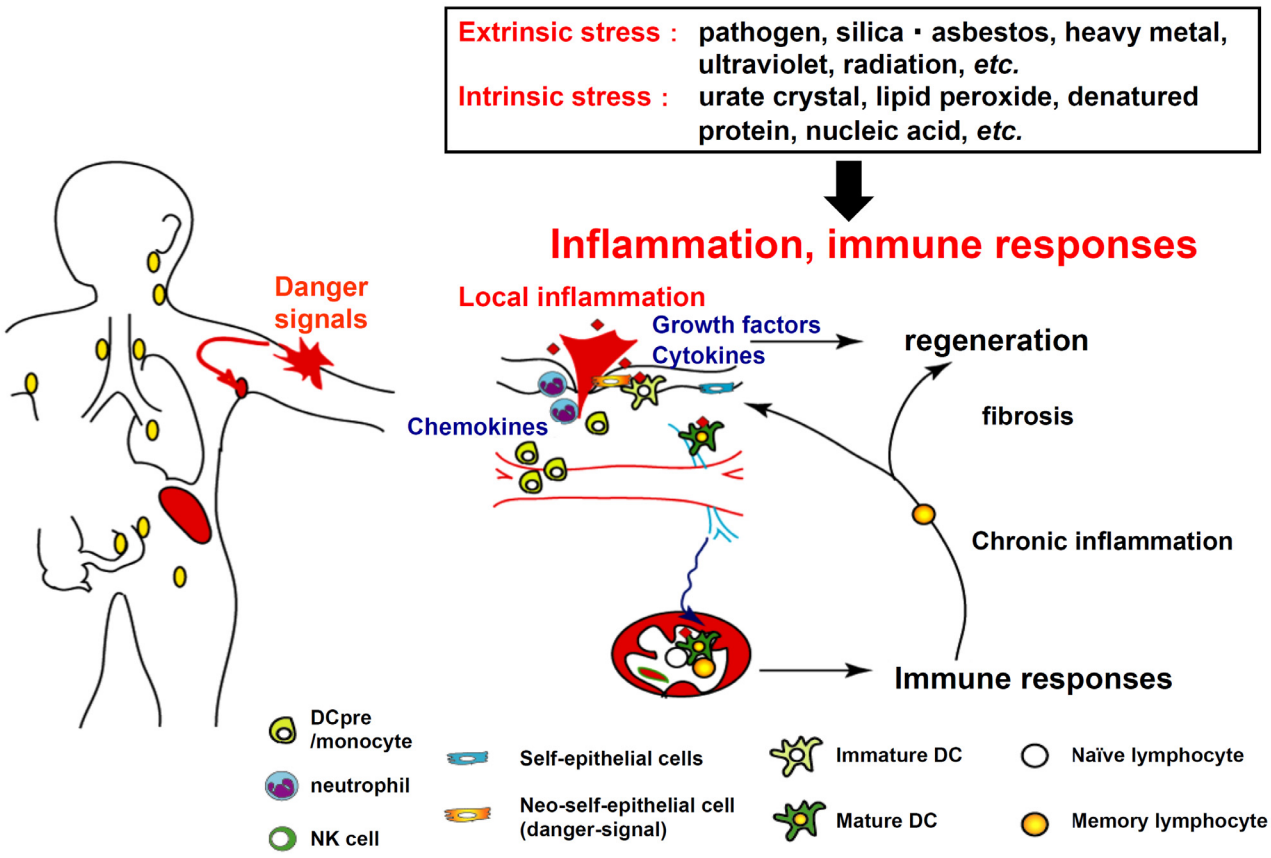

Fig.1. Summary of host defense against extrinsic and intrinsic stress.

Extrinsic and intrinsic stress induces local inflammation. Damaged epithelial cells produce cytokines, growth factors and chemokines as inflammatory mediators. Chemokines recruit circulating neutrophils, monocytes and DC precursors to the inflamed tissues as the first line of host defense (innate immunity). Tissue-infiltrating immature DCs capture tissue-associated antigens, differentiate to mature DCs, and further migrate to the draining-lymph nodes via afferent lymphatics. The lymph node migrating DCs activate antigen specific naïve and memory T cells. The activated T cells migrate to inflamed tissues via the circulation and provide the second line of host defense (acquired immunity).

The prototypic chemokines, a neutrophil chemotactic factor interleukin 8 (IL 8, CXCL8) and monocyte chemotactic factor MCAF/MCP-1 (CCL2) were purified and molecularly cloned by K.M. and T. Yoshimura in the late 1980's at National Cancer Institute, National Institutes of Health, USA[1,2]. Since the early 1970's, neutrophil and mono-

cyte chemotactic activities had been noticed in the conditioned media of activated leukocytes[3]. However, the substances responsible for these activities were not molecularly identified until our discovery of IL 8 and MCAF/MCP-1. Later,numerous related molecules termed chemokines were identified biochemically[4],through the signal trap method for the cDNA cloning[5] followed by the discovery in the data bases of the human genome[6,7]. The family of chemokines now comprises over 40 members, and is subdivided into 4 subfamilies based on the location of the first two cysteines in the molecule (Fig. 2). Mature forms of chemokines consist of around 70 amino acids, are very basic and bind to heparansulfate on proteoglycans. Twenty chemokine receptors have been identified to date, all of which belong to GPCRs associated with pertussis toxin-sensitive Gαi protein (Fig. 3). Chemokines usually form dimers or tetramers in solution[8], and their receptors also tend to form homodimers or occasionally heterodimers[9]. The physiological significance of homo/heterodimer formation by chemokine receptors remains elusive. There also exist so-called decoy chemokine receptors which negatively regulate inflammatory and immune responses[10]. In addition, some pathogens such as viruses[11] and ticks[12] produce molecules that mimick chemokines, chemokine receptors and antagonistic/agonistic molecules.

Chemokines and their receptors are considered promising targets for the regulation of leukocyte infiltration in inflammatory and immune diseases, and many pharmaceutical companies are currently conducting clinical development programs targeting the chemokine system.

Chemokine Receptor Signaling Pathways and FROUNT

Upon chemokine binding, like other chemoattractant receptors, the Gαβγ subunit dissociates from the receptor and the βγ subunit is released, which leads to a transient accumulation of phosphatidylinositol 3,4,5--trisphosphate (PIP3) at the leading edge of migrating cells that face the highest concentration of chemoattractant[13].This signaling is coupled to the activation of proteins containing PIP3 binding motifs pleckstrin homology (PH) domains and the DOCK homology region (DHR)-1[14]. The small G protein Rac is activated by PIP3 binding proteins and promotes F-actin polymerization and the formation of a lamellipodium protrusion at the cell front and maintain directional migration[15,16]. Nishikimi and Fukui identified that interactions between DOCK2 and the phospholipid, phosphatidic acid are required for stabilization of the leading edge of the cell and accurate chemotaxis[17]. However the initial amplifying steps in this signaling cascade, especially those immediately downstream of the chemokine receptor interaction, are yet to be fully elucidated.

The C-terminal domain of chemokine receptors, especially the membrane-proximal C-terminal region, has been reported to play an important role in the directional migration of cells. In experiments using various truncated mutants of CCR2 and CCR5, truncation at the membrane-proximal, but not distal, C-terminal region of these receptors abrogated cell migration[18,19]. As a molecule that illustrates the importance of this region for directional migration, we have demonstrated the critical role of FROUNT in both CCR2 and CCR5-mediated chemotaxis[20,21].FROUNT binds to the C-terminal of these receptors and promotes chemotaxis by upregulating PI3K activation. FROUNT also promotes the directionality of chemotaxis by amplifying signals in such a way as to form mainly one leading edge protrusion of pseudopodia toward higher chemokine concentrations (Fig. 4). FROUNT is also required for chemokine receptor clustering which is important for receptor signaling. This effect by FROUNT is G-protein-independent because pertussis toxin does not affect the formation of the chemokine receptor clusters. Thus, FROUNT is the factor that lies immediately downstream of chemokine-receptor interaction in chemotaxis signaling, and is a common regulator of both CCR2 and CCR5, controlling the directional migration of chemotaxis mediated by both these receptors. Functional suppression of FROUNT abbrogates infiltration of macrophages in an animal model of in vivo peritonitis. Recently, it was reported that mRNA levels of FROUNT are up-regulated in tissue samples from patients with heart failure and metastatic tumors[22,23].Inhibition of FROUNT expression prevents tumor cell migration and mesenchymal stem cell homing[23,24].

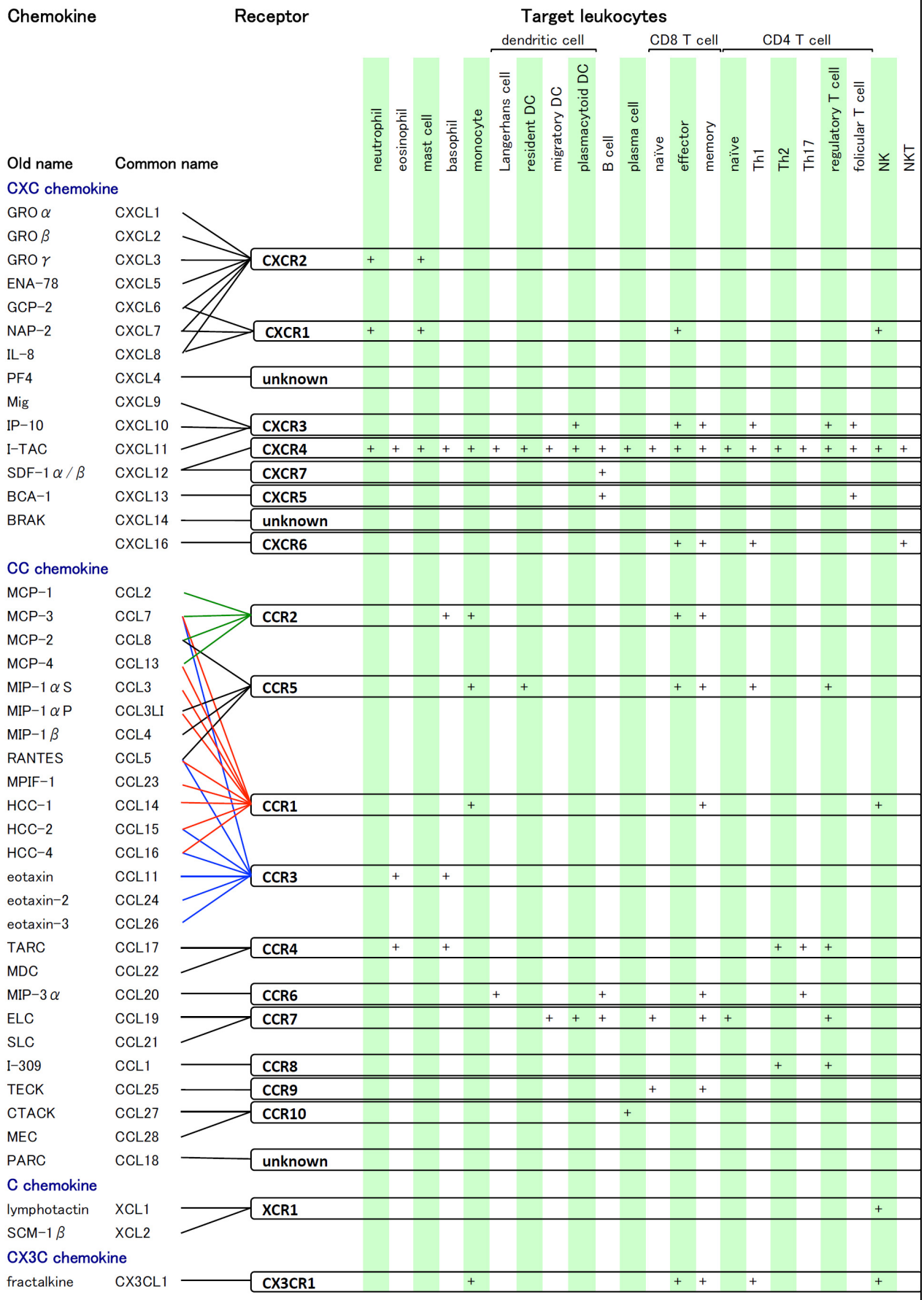

Fig.2. Chemokines and their receptors.

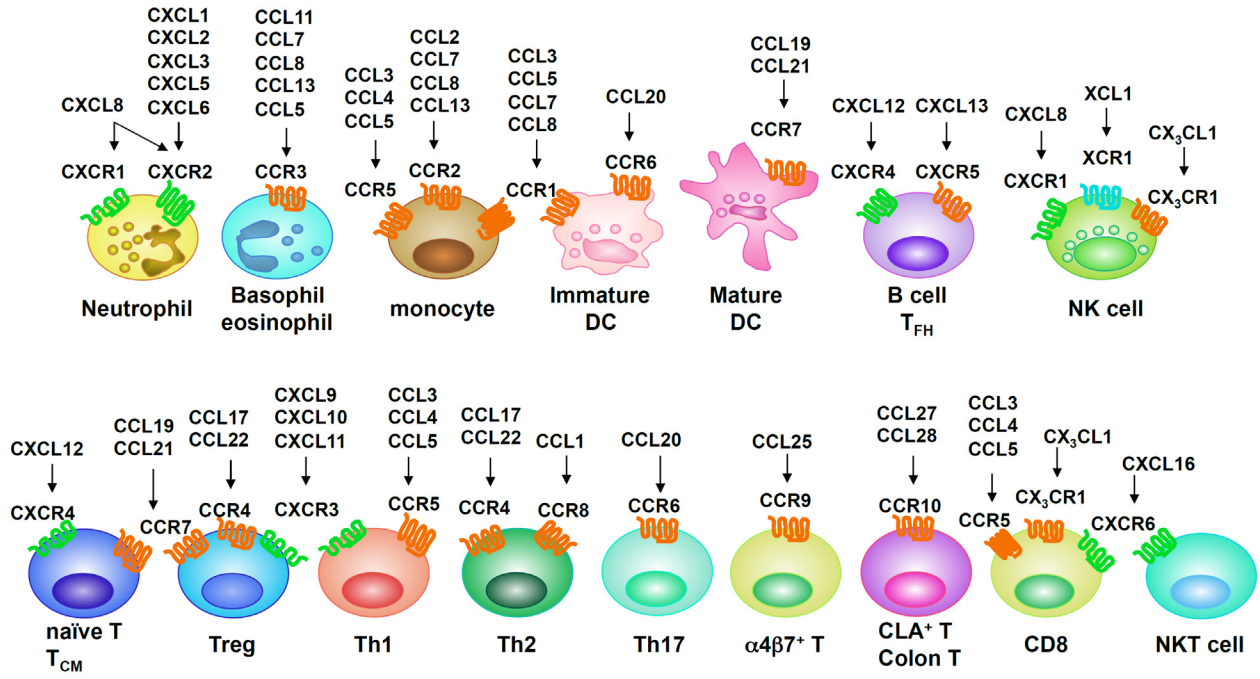

Fig.3. Major chemokine receptors expressed on leukocyte subsets.

Cyclophilin-A, Filamin-A, VASP, and LASP-1 are also chemokine recetor-interacting proteins that may modulate chemokine-mediated chemotaxis[25-28]. Inhibiting intracellular chemokine-activated signaling is a possible strategy for interfering with chemotaxis. Receptor-interacting proteins are potential therapeutic targets for the treatment of chemokine-related diseases.

Chemokines in inflammation

In acute inflammation such as that caused by bacterial infection, ischemia-reperfusion injury and acute glomerulonephritis, predominantly neutrophils infiltrate into the tissue. Critical role of CXCL8, related molecules, and their receptors CXCR1/2 has been established in various disease models[29]. The contribution of each ligand and the role of the receptors in different tissues and circumstances varies. Thus, dual specific antagonists for CXCR1/2 would be an ideal way to block neutrophil infiltration. When acute inflammation is unable to be resolved by neutrophils, the inflammation perpetuates and develops in chronic inflammation in which macrophages and lymphocytes predominate as the infiltrating cells. CCL2 and its related chemokines and their interaction with the common receptor CCR2 pivotally regulate macrophage infiltration[30]. Chronic bacterial infection such as tuberculosis and leprosy, chronic glomerulonephritis (IgA nephropathy, lupus nephritis and diabetic nephropathy), and arteriosclerosis are well known chronic inflammatory diseases. Typical histological features of chronic inflammation are granuloma formation together with fibrotic changes in which macrophages play a key role[31]. Obesity has recently become considered to be a chronic inflammatory disease, and macrophage infiltration into adipose tissues and the involvement of CCL2 in insulin resistance have attracted interest[32]. Furthermore, macrophages are key members of the so-called myeloid-derived suppressor cell subpopulation associated with tumors which is regulated by the CCL2-CCR2 signaling axis[33]. Therefore, CCR2 is a promising target for overcoming the immunosuppressive state in tumor bearing hosts.

Chemokines in lymphoid organogenesis and immunity

Forster and Lipp for the first time reported the critical involvement of the chemokine BLC (CXCL13) and its receptor CXCR5 in lymphoid organogenesis under physiological conditions[34]. The CXCL13-CXCR5 signaling axis is essential for recruiting B lymphocytes and Tfh in germinal center formation[35]. Since then, the critical role of CCR7 and its

ligand SLC (CCL21) in the migration of naïve T lymphocytes from the circulation into secondary lymph nodes through HEV[36] and in the migration of antigen captured dendritic cells from inflamed tissues into the draining lymph nodes have been observed[37].

The Th1 and Th2 paradigm has ruled the last two decades in immunology[38]. Two additional subsets of helper T lymphocytes, Th17[39] and Treg[40] have recently joined Th paradigm. As shown in Fig.5, each subset of Th cells preferentially expresses chemokine receptors which play an important role in the infiltration of that subset at specific immune sites. In addition, the mystery surrounding the molecular mechanism of the tissue specific homing properties of particular lymphocytes was solved recently by the discovery of the tissue imprinting of lymphocytes by Iwata et al[41]. Retinoic acid produced by dendritic cells in the mesenteric lymph node converts T lymphocytes to CCR9+α4β7+T lymphocytes, tissue-imprinted memory T lymphocytes that home to the intestinal lamina propria in a TECK (CCL25) and MAdCAM1-dependent manner.

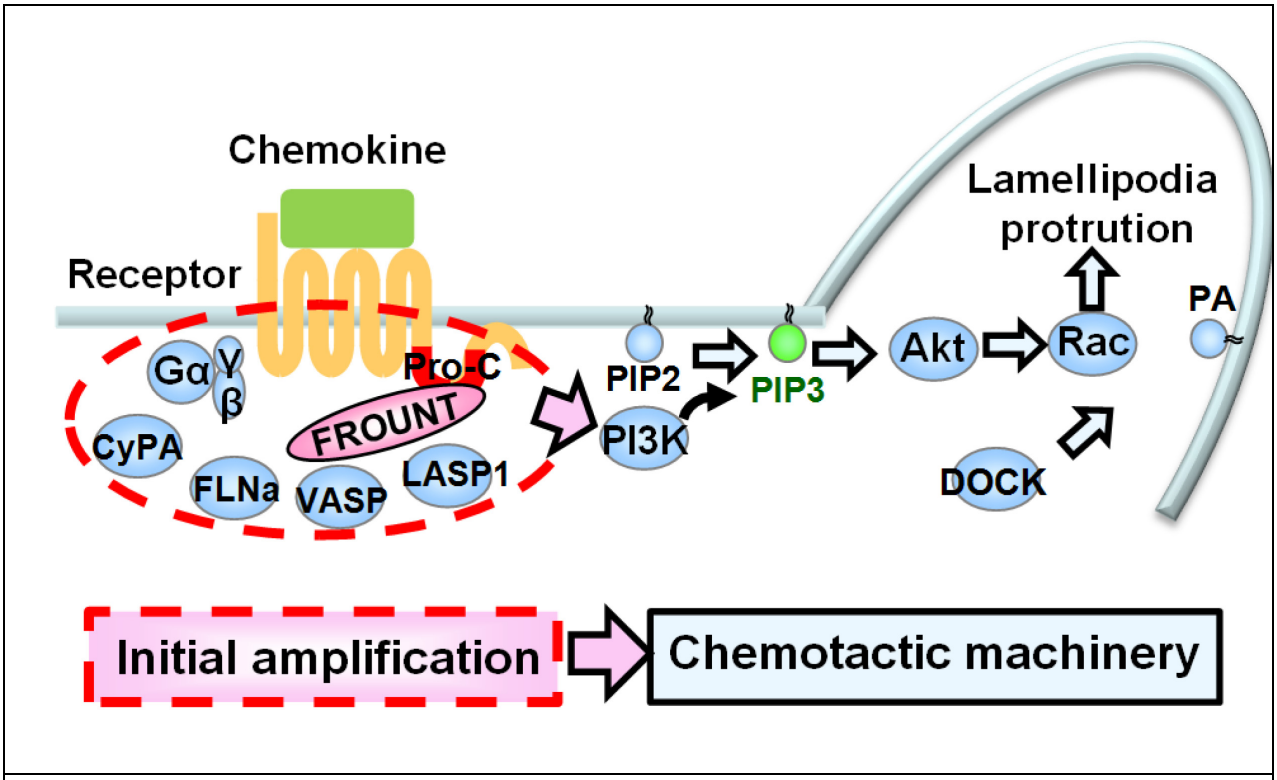

Fig.4. The chemokine induced intracellular signaling cascade in leukocyte chemotaxis.

FROUNT binds to the Pro-C (membrane-proximal C-terminal region of chemokine receptor), and activates the PI3K-PIP3-Akt-Rac-Lamellipodia protrution cascade. Gα(heterotrimeric G proteins), CyPA (Cyclophilin-A), FLNa (Filamin-A), VASP, and LASP-1 also interact directly with chemokine receptor.

CTL are major players in the eradication of virally infected cells and cancer. Chemokines play a critical role in the induction, tissue distribution and maintenance of CTL memory. We have recently found that CXCR3 regulates CD8+T cell differentiation into short-lived effector cells by the translocation of antigen stimulated T cells from the T cell zone to the marginal zone of the spleen during CTL memory induction. CXCR3, CXCR6 and CCR5 are considered to be the main receptors involved in the infiltration of effector CTL into tissues.

Chemokines and Cancer

CXC chemokines such as CXCL8 with an ELR motif just before the first cysteines promote angiogenesis, whereas CXC chemokines without an ELR motif inhibit angiogenesis[42]. Correlation between the CXCL8 production and tumor-associated angiogenesis in several human cancers has been reported[43]. In an experimental tumor model, the CXCL8 gene seems to be activated through ras activation,

while hypoxic conditions in the tumor induces VEGF[44]. Moreover, CXCL8 gene is very sensitive to oxidative stress, therefore a rational approach is to target CXCL8 and VEGF simultaneously in order to block tumor-associated angiogenesis.

Zlotonik et al. first described the possible involvement of chemokines in organ-specific cancer metastasis[45]. Furthermore, the CXCL12-CXCR4 signaling axis may regulate dissemination of gastric cancer cells into the peritoneal cavity[46]. CXCR7, another CXCL-12 receptor is specifically expressed on tumor-associated endothelium, and may promote tumor growth and metastasis. Additionally, some chemokines including CXCL8 and CXCL12 may directly activate tumor growth. In this sense, it is intriguing that CXCR1 is expressed on some cancer stem cells[47] and that CXCR2 could be a senescence marker[48].

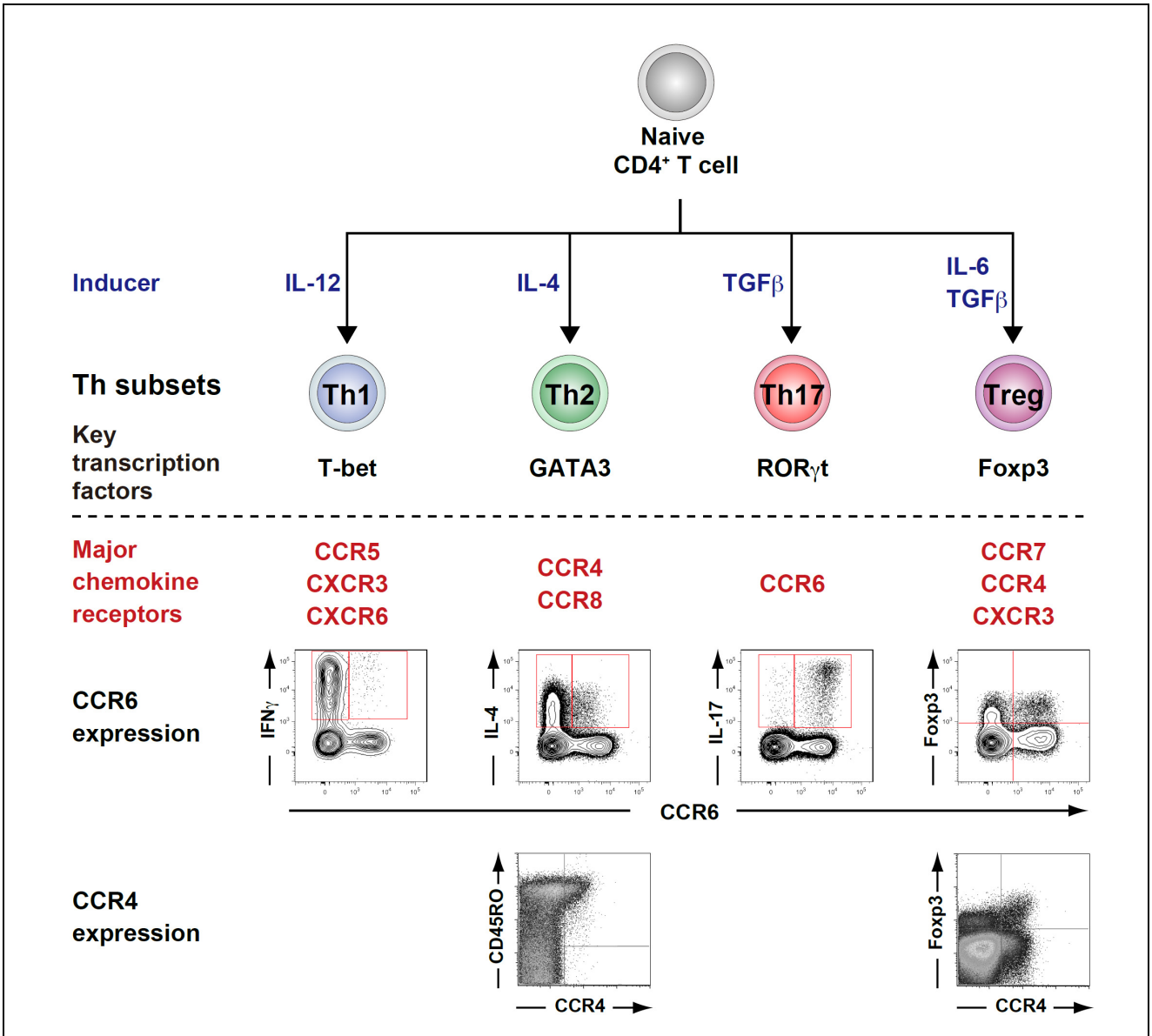

Fig. 5. CD4 Th subsets and chemokine receptor expressions.

Inducer, key transcription factors and major chemokine receptors of CD4 Th subsets. Flow-cytometry profiles represent expression of CCR6 and CCR4 on human CD4 T cell subsets.

Chemokine receptors as co-receptors for HIV infection

Berger et al. revealed that CXCR4 is a co-receptor for HIV to infect to CD4+ T lymphocytes[49]. Subsequently, it has been revealed that CCR5 acts as a co-receptor for HIV to enter CD4+ macrophages[50,51]. Through these studies, the long lasting mystery of the molecular mechanism for HIV infection of human leukocytes, and of the cellular tropism of HIV to either T lymphocytes or macrophages, were solved. Attachment of HIV to CD4 induces conformational change of gp120 of HIV, which is then used to bind co-receptors. The CCR5 deficient so-called delta32 homozygote or heterozygote occurs at a frequency of about 1% or 8% among Caucasian, respectively[51]. Interestingly, delta32 accumulates particularly in Northern Scandinavians. This deficiency is speculated to date back to the mid 14th century when the bubonic plague prevailed, a time when its CCR5 deficiency might have given people a survival advantage. Furthermore, CCR2V64I mutations, CXCL12 3' end mutations, CCL5 mutations, and CXCR1 haplotype[52] have also been reported to be involved in acquiring resistance to HIV infection.

ATLL and CCR4

Human adult T cell leukemia lymphoma (ATLL), and other T cell leukemia such as Sezary syndrome and Mycosis fungoides tend to infiltrate the skin. Yoshie et al. found that these malignant cells highly express CCR4[53], that HTLV-1 increases the expression of CCR4 in Fra2/JunD- and GATA3-dependent manner[54], and that HTLV-1 also induces the CCR4 MDC (CCL22) in order to recruit non-infected CCR4+CD4+ lymphocytes (Th2 or Treg) that accelerate virus transmission in a cell-to-cell contact-dependent manner. Downstream target genes of Fra2/JunD include c-Myb and SOX4 for growth promotion, and MDM2 for apoptosis inhibition[54]. Therefore, CCR4 is a bona fide tumor marker of ATLL, and a promising therapeutic target. K.M. collaborated with Nobuo Hanai in Kyowa Hakko Co. Ltd., Japan to produce a monoclonal antibody against human CCR4 through mouse hybridoma technology, which was subsequently converted to a humanized antibody with potent ADCC activity (KW-0761). A Phase II clinical trial using this humanized antibody for the treatment of recurrent ATLL has been completed in Japan revealing striking efficacy. Weekly administration of 1 mg/kg KW-0761 over 8 weeks resulted in 8 cases of complete remission and 5 cases of partial responses out of a total of 26 cases (overall response rate=50%)(American Society of Hematology, Dec. 4th-7th, 2010, Orland, FL, USA). The result of a Phase I clinical trial of KW-0761 has been published[55]. Similar efficacy has been also observed in Phase I/II clinical trials in USA for cutaneous lymphoma, Sezary syndrome and Mycosis fungoides (American Society of Hematology, Dec. 4th-7th, 2010, Orland, FL, USA). Furthermore, CCR4 is expressed on Treg as well as Th2 cells, therefore depletion of CCR4+Treg and Th2 by KW-0761 is expected to restore immune responses in immuno-suppressed cancer patients. In addition, KW-0761 for the treatment of allergic diseases has been licensed to Amgen Co, Ltd., USA from Kyowa Hakko Kirin Co. Ltd.

Chemotherapeutics and antibodies targeting chemokines and chemokine receptors

The CXCR1 antagonist, Repertaxin developed by Dompe Ltd., Italy is currently in a Phase II clinical trial targeting renal transplantation-associated reperfusion injury. Another CXCR1 antagonist, SCH527123 developed by Schering-Plough Ltd., USA, inhibits the release of myeloperoxidase as well as neutrophil chemotaxis in response to CXCL8 and is currently in a Phase II clinical trial for COPD.

CCR2 antagonists, and antibodies against CCR2 and its ligand CCL2 have been tested in the clinical trials for the treatment of osteoarthritis, Type II diabetes, arteriosclerosis, multiple sclerosis, and cancer. Furthermore, the development of CCR9 antagonist Traficet-EN for inflammatory bowel diseases by ChemoCentryx Ltd., USA appears to be processing well. Table 1 summarizes the current status of clinical trials of therapeutics targeting chemokines and their receptors.

Conclusion

The discovery of chemokines and advances in the characterization of leukocyte adhesion molecules have revolutionized our understanding of the molecular mechanisms of inflammation and immunity. In the past, in-

flammation research mostly depended on the examination of fixed pathological sections. However, it is now possible to visualize leukocyte trafficking in a real-time system in situ. Inflammatory and immune responses are now understood to be more spatially and temporally dynamic than previously thought. The development of therapeutics targeting chemokines and their receptors holds great promise for the treatment of intractable diseases in the near future.

Receptor	Development stage	Drug	Developer	Target diseases
CCR1	Phase I	CCX354	ChemoCentryx	Rheumatoid arthritis
CCR2	Phase II	CCX140	ChemoCentryx	Type II diabetes
	Phase II	BMS-741672	Bristol-Myers Squibb	Type II diabetes
	Phase II	MLN1202 (MAb)	Millennium/Takeda	Arteriosclerosis
	Phase II	PF-04136309	Pfizer	Osteoarthritis
	Phase I	INCB8696	Incyte	Multiple sclerosis
	Phase I	CNTO888 (MAb against CCL2)	Centocor, Inc.	Cancer
CCR3	Phase II	TPI ASM8	Topigen Pharmaceuticals	Asthma
CCR4	Phase I	KW-0761	Kyowa Hakko Kirin	Adult T-cell leukemia, peripheral T-cell lymphoma
CCR5	Approval	Maraviroc (CELSENTRI)	Pfizer	HIV infection
	Phase III	SCH417690 (Vicriviroc)	SchéringPlough	HIV infection
	Phase II	PF-00232798	Pfizer	HIV infection
	Phase II	PRO140(Ab)	Progenics Pharmaceuticals	HIV infection
	Phase II	INCB9471	Incyte	HIV infection
	Phase II	AZD5672	AstraZeneca	Rheumatoid arthritis
	Phase I/II	INCB15050	Incyte	HIV infection
	Phase I	CCR5mAb004	Human Genome Sciences	HIV infection
	Phase I	GSK706769	GSK	HIV infection
	Phase I	SCH532706	SchéringPlough	HIV infection
	Phase I	AZD8566	AstraZeneca	Rheumatoid arthritis
CCR7	Phase I	CMV-ALT	Duke University	Glioblastoma
	Phase I	DC/Apo-Nec	José Mordoh, MD, PhD	Melanoma
CCR9	Phase III	Traficet-EN	ChemoCentryx	Crohn's disease
	Phase II	Traficet-EN	ChemoCentryx	Celiac disease
	Phase I	Traficet-EN	ChemoCentryx	Ulcerative colitis
	Phase I	CCX025	ChemoCentryx	Gastrointestinal disease
CXCR1/2	Phase II	Repertaxin	Dompe	Transplantation, inflammatory bowel disease
	Preclinical	DF2156A	Dompe	Transplantation, inflammatory bowel disease
CXCR2	Phase II	SCH-527123	SchéringPlough	Chronic obstructive pulmonary disease
	Phase I	SB656933	GSK	Chronic obstructive pulmonary disease
CXCR3	Phase I	AS1409 (huBC1-huIL12)	Antisoma Research	Renal cell carcinoma, melanoma
	Preclinical	SCH 721015 with SCH 209702	SchéringPlough	Bladder cancer
CXCR4	Approval/ Phase II	AMD3100	Genzyme(AnorMED)	HIV infection
	Phase II/III	SP01A (Sphirewall)	Samaritan Pharmaceuticals	HIV infection
	Phase II	AMD070/AMD11070	Genzyme(AnorMED)	HIV infection
	Phase I	MSX-122	Metastatix	Cancer
	Phase I	TG-0054	TaiGen Biotechnology	Diabetic retinopathy
CXCR7	Preclinical	CXCR7	ChemoCentryx	Cancer

Table1
The current status of clinical trials of therapeutics targeting chemokines and their receptors

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