Angiogenesis 1998; 2; 123–134

REVIEW ARTICLE

CXC chemokines mechanism of action in regulating tumor angiogenesis

Bethany B. Moore, Douglas A. Arenberg, Christina L. Addison, Michael P. Keane, Peter J. Polverini[1] and Robert M. Strieter

Department of Internal Medicine (Division of Pulmonary and Critical Medicine), University of Michigan Medical School, Ann Arbor, MI 48109-0642,USA. [1]Section of Oral Pathology, University of Michigan Dental School, Ann Arbor, MI 48109-1078, USA

The CXC chemokines have recently been identified as a family of molecules which can regulate angiogenesis. Members of this family which contain the amino acid motif Glu–Leu–Arg in their amino terminus (ELR$^+$) act as angiogenic factors, while ELR$^-$ members act as angiostatic molecules. The balance of these angiogenic versus angiostatic factors is critical in regulating homeostasis. As we detail in this review, there is increasing evidence from a variety of tumor model systems to suggest that the angiogenic members of this family and their receptors may be playing an important role in the neovascular pathology of solid tumors. In contrast, the angiostatic effects of the ELR$^-$ family members may provide novel therapeutic strategies for treating many tumors. [© 1998 Kluwer Academic Publishers]

Key words: Angiogenesis, chemokines, chemotaxis, cytokines, neovascularization, tumorigenesis.

(Received 3 March 1998; accepted 7 May 1998)

The angiogenic process

Angiogenesis is the growth of new blood vessels from pre-existing vessels and capillaries. It is a biological process that is critical to a variety of physiological processes like embryogenesis and wound repair. However, it is also a process which can play a devastating role to the host in a number of pathological processes such as chronic inflammation, fibroproliferative disorders and also tumorigenesis.[1–9] The angiogenic response in the microvasculature is associated with changes in cellular adhesive interactions between adjacent endothelial cells, pericytes and surrounding extracellular matrix.[10–14] In the process of active neovascularization, endothelial cells reorganize their cytoskeleton, express cell surface adhesion molecules such as integrins and selectins, secrete proteolytic enzymes, and remodel their adjacent extracellular matrix.[15–23] These events are followed by the formation of capillary buds. Autocrine and/or paracrine angiogenic factors must be present to induce endothelial cell migration, proliferation, elongation, orientation and differentiation leading to the re-establishment of the basement membrane, lumen formation and anastomosis with other new or pre-existing vessels, eventually leading to the formation of intact microvessels.[18,24,25] If there is a preponderance of angiogenic factors in the local milieu, the neovasculature may persist as capillaries, or differentiate into mature venules or arterioles. If instead, the local milieu changes such that there is a preponderance of angiostatic factors, the neovessels can regress. The angiostatic factors which mediate regression can do so either by inducing apoptosis or cell cycle arrest of endothelial cells.[20,26,27] Thus the process of angiogenesis is regulated by a

Correspondence to R. M. Strieter, Department of Internal Medicine, Division of Pulmonary and Critical Care, 6200 MSRB III, 1150 West Medical Center Drive, University of Michigan Medical Center, Ann Arbor, MI 48109-0642, USA. Tel: (+1) 734 936-2612; Fax: (+1) 734 764-4556.

This work was supported, in part, by: NIH grants CA79046 (B. B. M.), CA72543 (D. A. A.), CA66180, P50 HL60289 and P50 HL46487 (R. M. S.), and an American lung association grant (D. A. A.).

complex balancing act between opposing angiogenic and angiostatic factors.

CXC chemokines

There have been a number of important angiogenic regulators described.[2,9] Perhaps the best studied of the angiogenic factors have been the molecules vascular endothelial growth factor (VEGF)[28,29] and basic fibroblast growth factor (bFGF).[30] In addition, angiostatin has been widely studied as an inhibitor of angiogenesis.[31] While there is no debating the importance of these factors in certain physiological processes (e.g. during embryogenesis), as well as during tumorigenesis, there is growing evidence that other angiogenic factors also play relevant roles. In 1992, a member of the CXC chemokine family, interleukin-8 (IL-8), was shown to be an angiogenic factor.[32–34] As will be discussed in this review, more recent work has shown that members of the CXC chemokine family can be divided into both promoters of angiogenesis, as well as inhibitors of angiogenesis. Chemokines can be made by all nucleated cells in the body, which makes them good candidates to be involved in a wide variety of different solid tumors.[35–48]

The human CXC chemokine family consists of chemotactic polypeptides that are less than 10 kDa and are characteristically heparin binding proteins. On the structural level, this family displays four highly conserved cysteine amino acid residues, with the first two cysteines separated by one non-conserved amino acid residue, hence the name CXC.[34–43] Although the CXC motif distinguishes this family from other chemokine families, a second structural domain within the CXC family members dictates their angiogenic potential (see below). The amino-terminus of the majority of the CXC chemokines contains three amino acid residues (Glu–Leu–Arg: the ELR motif) which preceed the first cysteine amino acid residue of the primary structure of these chemokines.[35–44] In general, those family members which contain the ELR motif have enhanced neutrophil binding capabilities and are angiogenic. In contrast, those members which do not contain the ELR motif are angiostatic. Table 1 lists some of the CXC chemokine family members which have been identified and separates them into whether they are angiogenic (ELR^+) or angiostatic (ELR^-) (reviewed elsewhere[35–37,39,44,49]). The angiogenic members include IL-8, epithelial neutrophil activating protein-78 (ENA-78), growth related genes (GRO-α, -β and -γ), granulocyte chemotactic protein-2 (GCP-2) and platelet basic protein (PBP) as well as its amino-terminal truncated forms which are generated by proteolytic cleavage with monocyte-derived proteases and include connective tissue activating protein-III (CTAP-III), β-thromboglobulin (β-TG) and neutrophil activating protein-2 (NAP-2).[50] GRO-α, -β and -γ are closely related CXC chemokines, with GRO-α originally described for its melanoma growth stimulatory activity.[51–53] IL-8, ENA-78 and GCP-2 were all initially identified on the basis of neutrophil activation and chemotaxis.[35–44]

The angiostatic members include platelet factor 4 (PF4), which was originally described

Table 1. The ELR^+ and ELR^- CXC chemokines, and their responses in the corneal micropocket model of angiogenesis when tested alone or in combination

Chemokine	Angiogenic response in the CMP assay
IL-8	++++
ENA-78	++++
GRO-α	++++
GRO-β	ND
GRO-γ	++++
GCP-2	++++
PBP	ND
CTAP-III	ND
β-TG	ND
NAP-2	ND
PF4	–
IP-10	–
MIG	–
SDF-1	–
IL-8 + IP-10	–
IL-8 + MIG	–
ENA-78 + IP-10	–
ENA-78 + MIG	–
bFGF + IP-10	–
bFGF + MIG	–
VEGF + IP-10	–
VEGF + MIG	–

In addition, the effect of the ELR^- CXC chemokines was tested on bFGF- and VEGF-induced angiogenesis. ND means not done in these experiments. In addition, the chemokines which score postitive in the CMP assay have also been determined to directly cause endothelial cell chemotaxis and proliferation.

for its ability to bind heparin and inactivate heparin's anticoagulation function.[54] Other ELR$^-$, angiostatic CXC chemokines include monokine-induced by interferon-γ (MIG),[43] interferon-γ-inducible protein (IP-10)[43] and stromal cell-derived factor (SDF-1). SDF-1 recently gained notoriety when it was shown that SDF-1 induces lymphocyte migration and prevents infection of T cells by lymphotropic strains of HIV-1.[43–48,55–67] Despite the name, IP-10 can be induced by all three interferons (IFN-α, -β and -γ), while MIG is unique in that it is only induced by IFN-γ. [43–48,55–67] Interestingly, although IFN-γ induces the production of the angiostatic molecules IP-10 and MIG, it attenuates expression of the angiogenic molecules IL-8, GRO-α and ENA-78.[68,69] This differential regulation of angiostatic versus angiogenic CXC chemokines by IFN-γ is likely to account for the previously documented angiostatic effects of IFN-γ on wound repair-associated angiogenesis.[2–4,70]

While numerous *in vivo* and *in vitro* investigations have shown the importance of CXC chemokines in inflammation as chemotactic/activating factors for neutrophils and mononuclear cells, only recently has it become apparent that these CXC chemokines are important in the regulation of angiogenesis.

CXC chemokines are potent regulators of angiogenesis

In 1982, Taylor showed that PF4 could produce an avascular zone when tested in a chorioallantoic membrane (CAM) assay.[71] In 1990, Maione and his colleagues had demonstrated that recombinant human PF4 (an ELR$^-$ CXC chemokine) could inhibit angiogenesis, and cause growth inhibition of melanoma and colon carcinomas.[71–74] While these were the first documented results showing that CXC chemokines could negatively regulate angiogenesis, it was not until 1992 that it was discovered that CXC chemokines could also positively mediate angiogenesis. IL-8, an ELR$^+$ CXC chemokine, was shown to be able to mediate both *in vitro* endothelial cell chemotactic and proliferative activity, as well as *in vivo* angiogenesis in the absence of preceding inflammation using bioassays of angiogenesis.[32–34] These experiments proved that IL-8 was having a direct effect on the endothelial cells and that this angiogenic activity was distinct from its ability to recruit neutrophils. These findings suggested that members of the CXC chemokine family function in a disparate manner and can behave as either potent angiogenic or angiostatic factors in regulating net neovascularization.

Based on these findings, we hypothesized that the 'ELR motif' of members of the CXC chemokine family is a structural/functional domain that dictates their angiogenic activity. To test this postulate, endothelial cell chemotaxis was performed in the presence or absence of varying concentrations of ELR$^+$ (IL-8 and ENA-78) or ELR$^-$ CXC chemokines (PF4 and IP-10) to ascertain whether CXC chemokines display disparate angiogenic activity. Both IL-8 and ENA-78 demonstrated a dose-dependent increase in endothelial migration with an EC_{50} of 5 nM.[49] In contrast, neither PF4 nor IP-10 induced significant endothelial cell chemotaxis.[49] Other CXC chemokines were tested for their ability to induce endothelial cell migration, including ELR$^+$ CXC chemokines GCP-2, GRO-α, GRO-β, GRO-γ, PBP, CTAP-III and NAP-2 or the ELR$^-$ CXC chemokine MIG. All of the ELR$^+$ CXC chemokines tested demonstrated significant endothelial cell chemotactic activity over background, whereas the endothelial cell chemotactic activity of MIG was similar to either PF4, IP-10 or background.[49] These studies established that CXC chemokines can be divided into two major groups (ELR$^+$ and ELR$^-$) with defined biological activities.

To confirm that PF4, IP-10 and MIG are inhibitors of angiogenesis, endothelial cell chemotaxis was performed in response to ELR$^+$ CXC chemokines (IL-8 or ENA-78) or bFGF with or without varying concentrations of PF4, IP-10 or MIG. The ELR$^-$ CXC chemokines (PF4, IP-10 or MIG) significantly inhibited endothelial cell migration in response to either the ELR$^+$ CXC chemokines or bFGF with an IC_{50} ranging from 50 pM to 1 nM.[49] In contrast to their ability to attenuate endothelial cell migration, neither IP-10 nor MIG inhibited IL-8-induced neutrophil chemotactic activity.[49]

These *in vitro* studies were extended to an *in vivo* angiogenesis bioassay. ELR^+ CXC chemokines (IL-8, ENA-78, GRO-α or GCP-2), bFGF or VEGF, either alone or in combination with ELR^- CXC chemokines (IP-10 or MIG) were implanted into a rat cornea using the cornea micropocket assay (CMP) of neovascularization.[49,76,77] The ELR^+ CXC chemokines (IL-8, ENA-78, GRO-α or GCP-2), bFGF or VEGF induced positive corneal angiogenic responses, whereas both IP-10 and MIG failed to induce a neovascular responses in the CMP. When combined with the ELR^+ CXC chemokines (IL-8, ENA-78, GRO-α or GCP-2), bFGF or VEGF, IP-10 markedly abrogated the ELR^+ CXC chemokine, bFGF, or VEGF-induced angiogenic response. Furthermore, MIG and SDF-1 similar to IP-10, inhibited IL-8-, ENA-78-, bFGF- and VEGF-induced corneal neovascularization (Table 1). The fact that both the ELR^+ and ELR^- CXC chemokines exerted their angiogenic or angiostatic effects, respectively, in the absence of cellular infiltration again suggests that the ability to modulate angiogenesis is independent of the role these molecules play as leukocyte chemoattractants.

To establish that the 'ELR motif' is the critical structural/functional domain that dictates angiogenic activity for members of the CXC chemokine family, site-directed mutants were constructed that contained either TVR (from IP-10) or DLQ (from PF4) amino acid residue substitutions for the 'ELR motif' of wild-type IL-8.[49] In addition a site-directed mutant of MIG was produced, containing the 'ELR motif' immediately adjacent to the first cysteine amino acid residue of the primary structure of MIG.[49] Endothelial cell chemotaxis was used to assess the biological activity of these muteins. Both IL-8 mutants failed to induce endothelial cell migration; however, both mutants inhibited the maximal endothelial chemotactic activity of wild-type IL-8.[49] Extending these studies to the CMP assay, both IL-8 muteins also inhibited the angiogenic response of either wild-type IL-8 or ENA-78.[49] In addition, both mutants inhibited bFGF-induced angiogenic activity in the CMP assay.[49] In contrast, the ELR^+ mutant of wild-type MIG induced a significant angiogenic response and wild-type MIG inhibited the angiogenic response of this mutant in the CMP assay.[49] These studies support the contention that the 'ELR' motif of CXC chemokines is the important structural/functional domain that dictates the angiogenic activity of CXC chemokines. Furthermore, these findings support the contention that ELR^- CXC chemokines are potent inhibitors of not only ELR^+ CXC chemokines, but also the unrelated angiogenic factors, bFGF and VEGF, a fact which could prove to be very useful therapeutically.

ELR^+ CXC chemokines regulate tumorigenesis

Folkman was the first to popularize the idea that tumors cannot grow beyond the size of 2 mm^3 in the absence of neovascularization and delivery of oxygen and nutrients.[1,4,78,79] Tumor-associated vascular mass can show as much as a 400% increase over normal tissue vascular mass.[4] Clearly then, an imbalance in the production of angiogenic, as compared to angiostatic, factors contributes to the pathogenesis of all solid tumor growth. Moreover, a number of studies have determined that the magnitude of tumor-derived angiogenesis is directly correlated with their metastatic potential.[80–86] This suggests that tumor-associated angiogenesis is somehow disregulated to allow the unchecked over-expression of the angiogenic factors or perhaps the inappropriate suppression of angiostatic ones.[4,87,88] Members of the CXC chemokine family have now been shown to be important regulators of tumorigenesis in many different tumor model systems.

IL-8 has been found to be significantly elevated in non-small cell lung cancer (NSCLC).[88] In addition, IL-8 was determined to be a major angiogenic factor contributing to overall tumor-derived angiogenic activity in NSCLC.[89,90] These studies were extended to an *in vivo* model system of human tumorigenesis, which employed a human NSCLC/SCID mouse chimera by injecting human NSCLC cell lines into SCID mice.[90] This model system demonstrated a progressive increase in tumor size that was directly correlated with tumor-derived IL-8[90] in both tumor homogenates and plasma. Interestingly, the histology of these

tumors demonstrated a paucity of infiltrating neutrophils despite an appropriate neutrophil chemotactic signal and the ability of murine neutrophils to respond to human IL-8.[91] The lack of tumor neutrophil infiltration is similar to the previous observation of IL-8 in association with freshly isolated human NSCLC tumors.[89] This paucity of neutrophils is probably best explained by the fact that high circulating levels of IL-8 diminish the ability of the neutrophils to sense a chemotactic gradient leading to the site of local production. This simulates the phenomenon recently described in the IL-8 transgenic mouse.[91] In these mice, human IL-8 overexpression is associated with increased circulating levels of IL-8, similar to what we have observed in the human NSCLC/SCID mice during tumorigenesis. The high circulating IL-8 levels correlated with a proportional decrease in L-selectin expression on neutrophils.[91] The IL-8 transgenic mice demonstrated a defect in neutrophil recruitment to the peritoneal cavity after i.p. injection of either exogenous IL-8 or thioglycollate.[91] The IL-8 transgenic mouse model exemplifies the importance of localized production of IL-8 in order to establish a chemotactic gradient and target leukocytes to sites of inflammation. Accordingly, our study establishes a potential mechanism whereby the tumor may evade an innate host response by releasing sufficient IL-8 into the circulation and attenuating neutrophil extravasation at the site of the tumor, yet at the same time promoting tumor-derived angiogenesis.

To ascertain whether IL-8 contributes to tumorigenesis of human NSCLC in SCID mice, animals were subjected to a strategy of IL-8 depletion. Tumor-bearing animals were either passively immunized with either neutralizing IL-8 or control antibodies, or not treated. Tumor-bearing animals treated with neutralizing antibodies to IL-8 demonstrated a greater than 40% reduction in tumor growth, that was paralleled by a reduction in spontaneous metastases to the lung.[90] To determine the mechanism of tumor growth inhibition, *ex vivo* angiogenic activity was evaluated from NSCLC tumor homogenates from animals that had been treated *in vivo* with either control or neutralizing anti-IL-8 antibodies. Eighty-five percent of tumor samples from control antibody-treated animals induced positive corneal angiogenic responses. In contrast, less than 33% of tumor samples from anti-IL-8-treated animals induced a positive corneal neovascular response. The decreased angiogenic activity also correlated with a reduction in tumor vessel density. These studies demonstrated that a primary angiogenic signal for NSCLC neovascularization *in vivo* was directly mediated by tumor-associated IL-8.

ELR$^-$ CXC chemokines regulate tumorigenesis

In an analogous manner, the angiostatic molecule IP-10 was shown to be an endogenous inhibitor of tumor formation. Squamous cell carcinomas, which in general are very slow growing lung cancers and grow poorly when xenotransplanted to SCID mice, were shown to have high endogenous levels of IP-10.[92] If a neutralizing antibody to IP-10 was given during the course of squamous cell tumor growth (modeled by injecting Calu 1 cells into SCID mice), tumor formation was increased.[92] Conversely, if recombinant IP-10 was given repeatedly to SCID mice injected with the aggressive A549 adenocarcinoma cell lines, tumor growth could be diminished by as much as 40%. The mechanism of growth inhibition by intratumor administration of recombinant human IP-10 was found to be correlated with a reduction in primary tumor-derived angiogenic activity and neovasculature. These studies demonstrate the existence of an imbalance in the over- and under-expression of angiogenic and angiostatic CXC chemokines, respectively, during tumorigenesis of NSCLC.

In a similar manner, work from Luan *et al*. has shown that GRO-α and -γ are critical oncogenic factors for melanoma.[93] This group was able to show by both message level and protein production, the expression of GRO-α in 70% of human melanoma lesions examined. Interestingly, immunohistochemical analysis revealed that GRO-α expression, as well as the expression of the putative GRO-α receptor (CXCR2) was present in both the tumor cells as well as the tumor-infiltrating endothelial cells of the microvessels in seven of the 11 tumors studied.[93] The biological consequence of this constitutive overexpression

was tested by transfecting the GRO-α, -β or -γ genes into mouse melan-a melanocytes, resulting in nearly 100% tumor formation when these clones were injected into SCID or Nude mice. In contrast, appropriate vector control transfections yielded tumors below 10% of the time. Furthermore, polyclonal antisera to GRO-α and -γ reduced tumor growth in the GRO-α and -γ transfected melan-a melanocyte tumor models, respectively.[92] Conditioned media from both the transfected cells was able to induce a neovascular response in the CMP assay of angiogenesis and, as expected, antibodies against these angiogenic CXC chemokine molecules inhibited this response, clearly implicating the overexpression of the GRO-α and -γ chemokines, as positive regulators of angiogenesis, in the pathogenesis of melanoma. This group went on to suggest a mechanism by which melanoma cells might be turning on the constitutive overexpression of the GRO chemokines.[92] In Hs294T melanoma cells, the half-life of the IκB protein is shortened in comparison to normal retinal epithelial cells. This results in constitutive NFκB localization to the nucleus in these cells. The authors propose that this constitutive NFκB activation, in concert with other constitutively active transcription factors (i.e. the 115 kDa IUR-binding factor), promotes constitutive expression of the GRO genes, resulting in an imbalance in the local production of angiogenic versus angiostatic factors. As one might predict, the expression of the angiostatic CXC chemokine IP-10 was very low in this system.[93]

Kitadai *et al*., working with human gastric carcinomas, examined IL-8 mRNA by Northern blot, *in situ* mRNA hybridization and IL-8 protein by ELISA in eight human gastric carcinoma cell lines and 39 gastric carcinomas.[94] High levels of IL-8 were found in six of eight gastric carcinoma cell lines and 32 of 39 gastric carcinoma specimens, as compared to normal mucosa control specimens. The levels of IL-8 in the neoplasms strongly correlated with the vascularity of the specimens as determined by vessel counting using antibodies to CD34 ($r = 0.812$, $p = 0.001$).[94] These data suggest that IL-8 produced by gastric tumor cells may regulate neovascularization, and hence the growth and metastasis of gastric carcinoma. In addition, Lingen *et al*. have demonstrated that IL-8 is the major inducer of neovascularization produced by oral squamous cell carcinomas.[95]

Finally, our unpublished observations working in a SCID model of human prostate cancer analogous to the one described above for NSCLC have shown that IL-8 is a positive regulator of tumor formation in SCID mice injected with the prostate cancer cell line PC-3. Conditioned media from this cell line is angiogenic in the CMP assay of angiogenesis and this activity is blocked in the presence of neutralizing antibody to IL-8. Furthermore, injection of neutralizing antibody to IL-8 reduced the growth of PC-3 tumors in SCID mice by 50%. In a similar manner, studies using another prostate cancer cell line, Du145 have shown that GRO-α, not IL-8, is the predominant CXC chemokine which regulates angiogenic activity in the CMP assay of angiogenesis. These studies highlight the fact that even within a particular type of tumor, different members of the CXC chemokine family can regulate angiogenic potential and tumorigenicity.

One important observation to note is that the ELR^+ CXC chemokines can exert their effect directly on the endothelial cells themselves. *In vitro* chemotaxis assays and the CMP assays all demonstrate that IL-8, ENA-78, GCP-2, PBP, CTAP-III, NAP-2, and GRO-α, -β and -γ have direct proliferative and chemotactic activities on the endothelial cells themselves.[49] Although GRO-α and -γ have been shown to be mitogenic for melanoma cells,[92] in the case of NSCLC and prostate cancer, we have clearly shown that the CXC chemokines are not having autocrine proliferative effects on the tumor cells themselves.

Chemokine receptors regulate angiogenesis

As mentioned earlier, the chemokines can be made by all nucleated somatic cells, and the receptors for these CXC chemokines have been cloned and identified to have specific ligand–receptor profiles. For example, IL-8 specifically binds to CXCR1, ELR^+ CXC chemokines bind to CXCR2, IP-10 and MIG (ELR^- CXC chem-

okines) bind to CXCR3, and SDF-1 binds to CXCR4 (Table 2).[44,96,97] Evidence suggests that the endothelial receptor for ELR$^+$ CXC chemokines is the CXC chemokine receptor 2, CXCR2. In support of this concept is the following information: (1) both IL-8 and NAP-2 (ELR$^+$ CXC chemokines) have been shown to bind endothelial cells,[97] (2) CXCR2 is the putative receptor for all ELR$^+$ CXC chemokines on neutrophils[42,98] and all ELR$^+$ CXC chemokines are angiogenic,[49] (3) while the Duffy antigen receptor for chemokines (DARC) has been identified on post-capillary venule endothelial cells,[101] this receptor binds not only ELR$^+$ CXC chemokines, but also a variety of CC chemokines without apparent signal-coupling,[101] (4) expression of CXCR2 in human burn wound (granulation tissue) is found in association with capillary endothelial cells in areas of neovascularization,[102] (5) expression of CXCR2 in human melanoma is associated with both melanoma cells and microvessels within the tumor,[93] and (6) recent findings from our laboratory have demonstrated that neutralizing antibodies to murine CXCR2 attenuate the angiogenic activity of murine KC (a structural and functional homolog of human GRO-α), murine MIP-2 (a structural and functional homolog of human GRO-β and-γ) and human IL-8 in the rat CMP bioassay, without inhibiting the angiogenic activity of either bFGF or VEGF (unpublished observation). While these observations support the notion that CXCR2 may be the receptor which mediates ELR$^+$ CXC chemokine-mediated angiogenesis, perhaps the most compelling evidence comes from the recent work studying the viral etiology of the extremely vascular angiosarcoma, Kaposi's sarcoma.[103–107]

Table 2. CXC chemokine receptors and some of their putative ligands

CXC chemokines	CXC chemokine receptors			
	CXCR1	CXCR2	CXCR3	CXCR4
IL-8	+	+	–	–
ENA-78	–[a]	+	–	–
GRO-α	–[a]	+	–	–
IP-10	–	–	+	–
MIG	–	–	+	–
SDF-1	–	–	+[b]	+

[a] Low-affinity binding.
[b] Unpublished observation

In 1994, Chang *et al*. discovered the Kaposi's sarcoma associated herpesvirus (KSHV or HHV-8).[108] This virus has been detected in every Kaposi's sarcoma biopsy, and is implicated in the pathogenesis of both Kaposi's sarcoma and of primary effusion B cell lymphomas (PELs).[108–113] The virus infects malignant and progenitor cells in these lesions, and encodes a number of putative oncogenes believed to be involved in the angiogenic pathogenesis of these diseases. ORF 74 of KSHV encodes a G-protein-coupled receptor (KSHV-GPCR) that stimulates signaling pathways leading to cell proliferation in a constitutive, agonist-independent manner.[103,104] KSHV-GPCR is most homologous to the human receptor for the angiogenic chemokine IL-8 or CXCR2.[103,104] Work by Bais *et al*. demonstrated that signaling through this KSHV-GPCR leads to transformation and tumorigenicity. They demonstrate that this constitutively active receptor activates two protein kinases, JNK/SAPK and p38MAPK, and triggers signaling cascades similar to inflammatory cytokines. Specifically, they suggest that the production of the transcription factor AP-1 leads to the activation and secretion of VEGF, which may in turn increase the angiogenic potential of the tumor.[104] Of interest, however, is that the IL-8 promoter is also strongly positively regulated by AP-1 (our unpublished observations)[40,112] and it would be interesting to see if constitutive activation of this 'CXCR2-like' receptor leads to the autocrine production of ELR$^+$ CXC chemokines. Regardless, the fact that a constitutively active CXCR2-like receptor has been pirated by a virus that induces an angiosarcoma supports the notion that CXCR2 is likely to be the 'angiogenic' receptor on endothelial cells and suggests that this might be an important therapeutic target to treat vascular neoplasms.

IP-10 appears to bind to a specific cell surface site on endothelial cells.[26] This receptor appears to be a heparin sulfate proteoglycan associated receptor, as either heparin together with IP-10 or heparinase pretreatment of endothelial cells attenuates IP-10 binding.[26] This binding site is specific for IP-10, as neither ELR$^+$ CXC chemokines nor various CC chemokines compete for binding on

this site. Furthermore, binding of IP-10 to endothelial cells results in inhibition of proliferation independent of calcium flux and apoptosis, and dependent on reversible cell cycle arrest.[26] It is not clear if this receptor represents the recently identified receptor for IP-10/MIG (CXCR3) expressed on T cells.[96] These findings suggest that IP-10 and potentially MIG are unique members of the CXC chemokine family that share a heparin sulfate proteoglycan component of their receptor that accounts for their binding to endothelial cells and subsequent angiostatic activity.

Conclusion

Angiogenesis is regulated by an opposing balance of angiogenic and angiostatic factors. The above studies have demonstrated that as a family, the CXC chemokines behave as either angiogenic or angiostatic factors, depending on the presence of the 'ELR motif' that immediately precedes the first cysteine amino acid residue of the primary structure of these cytokines. Moreover, CXC chemokines appear to be important endogenous factors that regulate angiogenesis in association with tumorigenesis in a number of different neoplasms. These findings support the notion that therapy directed at either inhibition of angiogenic or augmentation of angiostatic CXC chemokine biology may be a novel approach in the treatment of solid tumors.

References

1. Folkman J, Cotran R. Relation of vascular proliferation to tumor growth. *Int Rev Exp Pathol* 1976; **16**, 207–248.
2. Folkman J, Klagsbrun M. Angiogenic factors. *Science* 1987; **235**, 442–447.
3. Folkman J, Brem H. Angiogenesis and inflammation. In: Gallin JI, Goldstein IM, Snyderman R (eds), *Inflammation: Basic Principles and Clinical Correlates*, 2nd edn. New York: Raven Press 1992: 821–839.
4. Folkman J. Tumor angiogenesis. In: Holland JF, III EF, Jr RCB, Kufe DW, Morton DL, Weischelbaum RR (eds), *Cancer Medicine*, 3rd edn. Philadelphia: Lea & Febiger 1993: 153–170.
5. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med* 1995; **1**, 27–31.
6. Polverini PJ, Cotran PS, Gimbrone MA, Unanue ER. Activated macrophages induce vascular proliferation. *Nature* 1977; **269**, 804–806.
7. Polverini PJ. How the extracellular matrix and macrophages contribute to angiogenesis-dependent diseases. *Eur J Cancer* 1996; **32A**, 2430–2437.
8. Leibovich SJ, Weisman DM. Macrophages, wound repair and angiogenesis. *Prog Clin Biol Res* 1988; **266**, 131–145.
9. Auerbach R. *Angiogenesis-inducing Factors: A Review*. New York: Academic Press 1981.
10. Phillips GD, Whitehead RA, Knighton DR. Initiation and pattern of angiogenesis in wound healing in the rat. *Am J Anat* 1991; **192**, 257–262.
11. Ausprunk DH, Folkman J. Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumor angiogenesis. *Microvasc Res* 1977; **14**, 53–65.
12. Ausprunk DH, Folkman J. The sequence of events in the regression of corneal capillaries. *Lab Invest* 1978; **38**, 284–296.
13. Ausprunk DH, Knighton DR, Folkman J. Differentiation of vascular endothelium in the chick chorioallantois: a structure and autoradiographic study. *Dev Biol* 1974; **38**, 237–248.
14. Sims DE. The pericyte: a review. *Tissue Cell* 1986; **18**, 153–174.
15. Madri JA, Pratt BM, Tucker AM. Phenotypic modulation of endothelial cells by transforming growth factor-beta depends upon the composition and organization of the extracellular matrix. *J Cell Biol* 1988; **106**, 1375–1384.
16. Ingber DE, Folkman J. Mechanochemical switching between growth and differentiation during fibroblast growth factor-stimulated angiogenesis *in vitro*: role of extracellular matrix. *J Cell Biol* 1989; **109**, 317–330.
17. Ingber DE. Extracellular matrix and cell shape: potential control points for the inhibition of angiogenesis. *J Cell Biochem* 1991; **47**, 236–241.
18. Gamble JR, Matthias U, Meyer G. Regulation of *in vitro* capillary tube formation by anti-integrin antibodies. *J Cell Biol* 1993; **121**, 921–943.
19. Nguyen M, Strubel NA, Bischoff J. A role for Lewis-X/A glycoconjugates in capillary morphogenesis. *Nature* 1993; **365**, 267–269.
20. Brooks PC, Montgomery AM, Rosenfeld M, *et al*. Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell* 1994; **79**, 1157–1164.
21. Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin alpha v beta 3 for angiogenesis. *Science* 1994; **264**, 569–571.
22. Rifkin DB, Gross JL, Moscatelli D, Jaffe E. Proteases and angiogenesis: production of plasminogen acti-

vator and collagenase by endothelial cells. In: Nossel H, Vogel HJ (eds), *Pathobiology of the Endothelial Cell*. New York: Academic Press 1982: 191–197.
23. Pepper MS, Vassaui JD, Orci L, Montesano R. Proteolytic balance and capillary morphogenesis *in vitro*. In: Steiner R, Weisz PB, Langer R (eds), *Angiogenesis: Key Principles*. Basel: Birkhauser Verlag 1992: 137–145.
24. Konerding MA, VanAckern C, Steinberg F, Streffer C. Combined morphological approaches in the study of network formation in tumor angiogenesis. In: Steiner R, Weisz PB, Langer R (eds), *Angiogenesis: Key Principles*. Basel: Birkhauser Verlag 1992: 40–58.
25. Paweletz N, Knierim M. Tumor-related angiogenesis. *Crit Rev Oncol Hematol* 1989; **9**, 197–242.
26. Luster AD, Greenberg SM, Leder P. The IP-10 chemokine binds to a specific cell surface heparan sulfate shared with platelet factor 4 and inhibits endothelial cell proliferation. *J Exp Med* 1995; **182**, 219–232.
27. Re F, Zanetti A, Sironi M, *et al*. Inhibition of anchorage-dependent cell spreading triggers apoptosis in cultured human endothelial cells. *J Cell Biol* 1994; **127**, 537–546.
28. Ferrara N. Vascular endothelial growth factor. *Eur J Cancer* 1996; **14**, 2413–2422.
29. Hanahan D, Folkman J. Pattens and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996; **86**, 356–364.
30. Rogelj S, Klagsbrun M, Atzmon R, *et al*. Basic fibroblast growth factor is an extracellular matrix component required for supporting the proliferation of vascular endothelial cells and the differentiation of PC12 cells. *J Cell Biol* 1989; **109**, 823–831.
31. O'Reilly MS, Holmgren L, Shing Y, *et al*. Angiostatin: a novel angiogenesis inhibitor that mediates suppression of metastases by a Lewis lung carcinoma. *Cell* 1994; **79**, 315–328.
32. Koch AE, Polverini PJ, Kunkel SL, *et al*. Interleukin-8 (IL-8) as a macrophage-derived mediator of angiogenesis. *Science* 1992; **258**, 1798–1801.
33. Strieter RM, Kunkel SL, Elner VM, *et al*. Interleukin-8: A corneal factor that induces neovascularization. *Am J Pathol* 1992; **141**, 1279–1274.
34. Hu DE, Hori Y, Fan T-P. Interleukin 8 stimulates angiogenesis in rats. *Inflammation* 1993; **17**, 135–143.
35. Walz A, Kunkel SL, Strieter RM. CXC chemokines—an overview. In: Koch AE, Strieter RM (eds), *Chemokines in Disease*. Austin, TX: R. G. Landes 1996: 1–26.
36. Strieter RM, Kunkel SL. Chemokines and the lung. In: Crystal R, West J, Weibel E, *et al*. (eds), *Lung: Scientific Foundations*, 2nd edn. New York: Raven Press 1997: 155–186.
37. Strieter RM, Lukacs NW, Standiford TJ, Kunkel SL. Cytokines and lung inflammation. *Thorax* 1993; **48**, 765–769.
38. Baggiolini M, Walz A, Kunkel SL. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. *J Clin Invest* 1989; **84**, 1045–1049.
39. Baggiolini M, Dewald B, Walz A. Interleukin-8 and related chemotactic cytokines. In: Gallin JI, Goldstein IM, Snyderman R (eds), *Inflammation: Basic Principles and Clinical Correlates*. New York: Raven Press 1992.
40. Matsushima K, Oppenheim JJ. Interleukin 8 and MCAF: novel inflammatory cytokines inducible by IL-1 and TNF. *Cytokine* 1989; **1**, 2–13.
41. Miller MD, Krangel MS. Biology and biochemistry of the chemokines: a family of chemotactic and inflammatory cytokines. *Crit Rev Immunol* 1992; **12**, 17–46.
42. Taub DD, Oppenheim JJ. Chemokines, inflammation and immune system. *Therapeutic Immunol* 1994; **1**, 229–246.
43. Farber JM. Mig and IP-10: CXC chemokines that target lymphocytes. *J Leuk Biol* 1997; **61**, 246–257.
44. Rollins BJ. Chemokines. *Blood* 1997; **90**, 909–928.
45. Bleul CC, Farzan M, Choe H, *et al*. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature* 1996; **382**, 829–833.
46. Oberlin E, Amara A, Bachelerie F, *et al*. The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. *Nature* 1996; **382**, 833–835.
47. Tashiro K, Tada H, Heilker R, Shirozu M, Nakano T, Honjo T. Signal sequence trap: a cloning strategy for secreted proteins and type I membrane proteins. *Science* 1993; **261**, 600–603.
48. Shirozu M, Nakano T, Inazawa J, *et al*. Structure and chromosomal localization of the human stromal cell-derived factor 1 (SDF-1) gene. *Genomics* 1995; **28**, 495–500.
49. Strieter RM, Polverini PJ, Kunkel SL, *et al*. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. *J Biol Chem* 1995; **270**, 27348–27357.
50. Walz A, Baggiolini M. Generation of the neutrophil-activating peptide NAP-2 from platelet basic protein or connective tissue-activating peptide III through monocyte proteases. *Exp Med* 1990; **171**, 449–454.
51. Ansiowicz A, Bardwell L, Sager R. Constitutive overexpression of a growth-regulated gene in

transformed Chinese hamster and human cells. *Proc Natl Acad Sci USA* 1987; **84**, 7188–7192.
52. Ansiowicz A, Zajchowski D, Stenman G, Sager R. Functional diversity of *gro* gene expression in human fibroblasts and mammary epithelial cells. *Proc Natl Acad Sci USA* 1988; **85**, 9645–9649.
53. Richmond A, Thomas HG. Melanoma growth stimulatory activity: isolation from human melanoma tumors and characterization of tissue distribution. *J Cell Biochem* 1988; **36**, 185–198.
54. Deutsch E, Kain W. Studies on platelet factor 4. In: Jonson SA, Monto RW, Rebuck JW, *et al*. (eds), *Blood Platelets*. Boston: Little-Brown 1961.
55. D'Apuzzo M, Rolink A, Loetscher M, *et al*. The chemokine SDF-1, stromal cell-derived factor 1, attracts early stage B cell precursors via the chemokine receptor CXCR4. *Eur J Immunol* 1997; **27**, 1788–1793.
56. Amara A, Gall SL, Schwartz O, *et al*. HIV coreceptor downregulation as antiviral principle: SDF-1alpha-dependent internalization of the chemokine receptor CXCR4 contributes to inhibition of HIV replication. *J Exp Med* 1997; **186**, 139–146.
57. Farber JM. HuMIG: a new member of the chemokine family of cytokines. *Biochem Biophys Res Commun* 1993; **192**, 223–230.
58. Vanguri P, Farber JM. Identification of CRG-2. An interferon-inducible mRNA predicted to encode a murine monokine. *J Biol Chem* 1990; **265**, 15049–15057.
59. Luster AD, Weinshank RL, Feinman R, Ravetch JV. Molecular and biochemical characterization of a novel gamma-interferon-inducible protein. *J Biol Chem* 1988; **263**, 12036–12043.
60. Luster AD, Unkeless JC, Ravetch JV. Gamma-interferon transcriptionally regulates an early-response gene containing homology to platelet proteins. *Nature* 1985; **315**, 672–676.
60. Luster AD, Jhanwar SC, Chaganti RS, Kersey JH, Ravetch JV. Interferon-inducible gene maps to a chromosomal band associated with a (4;11) translocation in acute leukemia cells. *Proc Natl Acad Sci USA* 1987; **84**, 2868–2871.
62. Farber JM. A macrophage mRNA selectively induced by gamma-interferon encodes a member of the platelet factor 4 family of cytokines. *Proc Natl Acad Sci USA* 1990; **87**, 5238–5242.
63. Farber JM. A collection of mRNA species that are inducible in the RAW 264.7 mouse macrophage cell line by gamma interferon and other agents. *Mol Cell Biol* 1992; **12**, 1535–1545.
64. Luster AD, Ravetch JV. Genomic characterization of a gamma-interferon-inducible gene (IP-10) and identification of an interferon-inducible hypersensitive site. *Mol Cell Biol* 1987; **7**, 3723–3731.
65. Liao F, Rabin RL, Yannelli JR, Koniaris LG, Vanguri P, Farber JM. Human Mig chemokine: biochemical and functional characterization. *J Exp Med* 1995; **182**, 1301–1314.
66. Lee HH, Farber JM. Localization of the gene for the human MIG cytokine on chromosome 4q21 adjacent to INP10 reveals a chemokine 'mini-cluster'. *Cytogenet Cell Genet* 1996; **74**, 255–258.
67. Amichay D, Gazzinelli RT, Karupiah G, Moench TR, Sher A, Farber JM. Genes for chemokines Mu-Mig and Crg-2 are induced in protozoan and viral infections in response to IFN-gamma with patterns of tissue expression that suggest nonredundant roles *in vivo*. *J. Immunol.* 1996; **157**, 4511–4520.
68. Gusella GL, Musso T, Bosco MC, Espinoza-Delgado I, Matsushima K, Varesio L. IL-2 up-regulates but IFN-γ suppresses IL-8 expression in human monocytes. *J Immunol* 1993; **151**, 2725–2732.
69. Schnyder-Candrian S, Strieter RM, Kunkel SL, A. AW. Interferon-α and interferon-γ downregulate the production of interleukin-8 and ENA-78 in human monocytes. *J Leuk Biol* 1995; in press.
70. Klagsbrun M, D'Amore PA. Regulators of angiogenesis. *Annu Rev Physiol* 1991; **53**, 217–239.
71. Taylor S, Folkman J. Protamine is an inhibitor of angiogenesis. *Nature* 1982; **297**, 307–312.
72. Maione TE, Gray GS, Petro J, *et al*. Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides. *Science* 1990; **247**, 77–79.
73. Maione TE, Gray GS, Hunt AJ, Sharpe RJ. Inhibition of tumor growth in mice by an analogue of platelet factor 4 that lacks affinity for heparin and retains potent angiostatic activity. *Cancer Res* 1991; **51**, 2077–2083.
74. Hansell P, Maione TE, Borgstrom P. Selective binding of platelet factor 4 to regions of active angiogenesis *in vivo*. *Am J Physiol* 1995; **269**, H829–836.
75. Sharpe RJ, Byers HR, Scott CF, Bauer SI, Maione TE. Growth inhibition of murine melanoma and human colon carcinoma by recombinant human platelet factor 4. *J Natl Cancer Inst* 1990; **82**, 848–853.
76. Strieter RM, Kunkel SL, Arenberg DA, Burdick MD, Polverini PJ. Interferon gamma-inducible protein 10 (IP-10), a member of the C-X-C chemokine family, is an inhibitor of angiogenesis. *Biochem Biophys Res Commun* 1995; **210**, 51–57.
77. Arenberg DA, Polverini PJ, Kunkel SL, Shanafelt A, Strieter RM. *In vitro* and *in vivo* systems to assess the role of CXC chemokines in the regulation of angiogenesis. *Methods Enzymol* 1997; **283**, 190–220.
78. Bouck N. Tumor angiogenesis: the role of oncogenes and tumor suppressor genes. *Cancer Cells* 1990; **2**, 179–185.

79. Bouck N. Angiogenesis: a mechanism by which oncogenes and tumor suppressor genes regulate tumorigenesis. *Cancer Treat Res* 1992; **63**, 359–371.
80. Folkman J, Watson K, Ingber D, Hanahan D. Induction of angiogenesis during the transition from hyperplasia to neoplasia. *Nature* 1989; **339**, 58–61.
81. Maiorana A, Gullino PM. Acquisition of angiogenic capacity and neoplastic transformation in the rat mammary gland. *Cancer Res* 1978; **38**, 4409–4414.
82. Herlyn M, Clark WH, Rodeck U, Manciati ML, Jambrosic J, Koprowski H. Biology of tumor progression in human melanocytes. *Lab Invest* 1987; **56**, 461–474.
83. Weidner N. Intratumor microvessel density as a prognostic factor in cancer. *Am J Pathol* 1995; **147**, 9–19.
84. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis—correlation in invasive breast carcinoma. *N Engl J Med* 1991; **324**, 1–8.
85. Weidner N, Carroll PR, Flax J, Blumenfeld W, Folkman J. Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. *Am J Pathol* 1993; **143**, 401–409.
86. Macchiarini P, Fontanini G, Hardin MJ, Squartini F, Angeletti CA. Relation of neovascularization to metastasis of non-small cell lung cancer. *Lancet* 1992; **340**, 145–146.
87. Folkman J. Clinical applications of research on angiogenesis. *N Eng J Med* 1995; **333**, 1757–1763.
88. Eisenstein R, Kuettner KE, Neopolitan C, Sobel LW, Sorgente N. The resistance of certain tissues to invasion III. Cartilage extracts inhibit the growth of fibroblasts and endothelial cells in culture. *Am J Pathol* 1975; **81**, 337–347.
89. Smith DR, Polverini PJ, Kunkel SL, *et al*. Inhibition of IL-8 attenuates angiogenesis in bronchogenic carcinoma. *J Exp Med* 1994; **179**, 1409–1415.
90. Arenberg DA, Kunkel SL, Polverini PJ, Glass M, Burdick MD, Strieter RM. Inhibition of interleukin-8 reduces tumorigenesis of human non-small cell lung cancer in SCID mice. *J Clin Invest* 1996; **97**, 2792–2802.
91. Simonet WS, Hughes TM, Nguyen HQ, Trebasky LD, Danilenko DM, Medlock ES. Long term impaired neutrophil migration in mice overexpressing human interleukin-8. *J Clin Invest* 1994; **94**, 1310–1319.
92. Arenberg DA, Kunkel SL, Polverini PJ, *et al*. Interferon-γ-inducible protein 10 (IP-10) is an angiostatic factor that inhibits human non-small cell lung cancer (NSCLC) tumorigenesis and spontaneous metastases. *J Exp Med* 1996; **184**, 981–992.
93. Luan J, Shattuck-Brandt R, Haghnegahdar H, *et al.* Mechanism and biological significance of constitutive expression of MGSA/GRO chemokines in malignant melanoma tumor progression. *J Leuk Biol* 1997; **62**, 588–597.
94. Kitadai Y, Haruma K, Sumii K, *et al*. Expression of interleukin-8 correlates with vascularity in human gastric carcinomas. *Am J Pathol* 1998; **152**, 93–100.
95. Lingen MW, Polverini PJ, Bouck NP. Retinoic acid induces cells cultured from oral squamous cell carcinomas to become anti-angiogenic. *Am J Pathol* 1996; **149**, 247–258.
96. Loetscher M, Gerber B, Loetscher P, *et al.* Chemokine receptor specific for IP10 and mig: structure, function, and expression in activated T-lymphocytes. *J Exp Med* 1996; **184**, 963–969.
97. Premack BA, Schall TJ. Chemokine receptors: gateways to inflammation and infection. *Nat Med* 1996; **2**, 1174–1178.
98. Schönbeck U, Brandt E, Petersen F, Flad H, Loppnow H. IL-8 specifically binds to endothelial but not to smooth muscle cells. *J Immunol* 1995; **154**, 2375–2383.
99. Lee J, Horuk R, Rice GC, Bennett GL, Camerato T, Wood WI. Characterization of two high affinity human interleukin-8 receptors. *J Biol Chem* 1992; **267**, 16283–16287.
100. Hadley TJ, Lu Z, Wasniowska K, Peiper SC, Hesselgesser J, Horuk R. Postcapillary venule endothelial cells in kidney express a multispecific chemokine receptor that is structurally and functionally identical to the erythroid isoform, which is the Duffy bloodgroup antigen. *J Clin Invest* 1994; **94**, 985–991.
101. Neote K, Darbonne W, Ogez J, Horuk R, Schall T. Identification of a promiscuous inflammatory peptide receptor on the surface of red blood cells. *J Biol Chem* 1993; **268**, 985–991.
102. Nanney LB, Mueller SG, Bueno R, Peiper SC, Richmond A. Distributions of melanoma growth stimulatory activity or growth-related gene and the interleukin-8 receptor type B in human wound repair. *Am J Pathol* 1995; **147**, 1248–1260.
103. Arvanitakis L, Geras-Raaka E, Varma A, Gershengorn MC, Cesarman E. Human herpesvirus KSHV encodes a constitutively active G-protein-coupled receptor linked to cell proliferation [see comments]. *Nature* 1997; **385**, 347–350.
104. Bais C, Santomasso B, Coso O, *et al*. G-protein-coupled receptor of Kaposi's sarcoma-associated herpesvirus is a viral oncogene and angiogenesis activator. *Nature* 1998; **391**, 86–89.
105. Boshoff C. Coupling herpesvirus to angiogenesis. *Nature* 1998; **391**, 24–25.
106. Boshoff C, Endo Y, Collins PD, *et al.* Angiogenic and HIV-inhibitory functions of KSHV-encoded chemokines. *Science* 1997; **278**, 290–294.

107. Moore PS, Boshoff C, Weiss RA, Chang Y. Molecular mimicry of human cytokine and cytokine response pathway genes by KSHV. *Science* 1996; **274**, 1739–1744.
108. Chang Y, EC, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposis's sarcoma. *Science* 1994; **266**, 1865–1869.
109. Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM. Kaposi's sarcoma-associated herpesvirus-like DNA sequences are present in AIDS-related body cabity B-cell lymphoma. *N Engl J Med* 1995; **335**, 1186–1191.
110. Boshoff C, Weiss RA. Kaposis sarcoma associated herpesvirus. *Curr Opin Infect Dis* 1997; **10**, 26–31.
111. Russo JJ, Bohenzky RA, Chien MC, *et al*. Nucleotide sequence of the Kaposi's sarcoma-associated herpesvirus (HHV8). *Proc Natl Acad Sci USA* 1997; **93**, 14862–14867.
112. Renne R, Zhong W, Herndier B, *et al*. Lytic growth of Kaposi's sarcoma associated herpesvirus (HHV-8) in culture. *Nat Med* 1996; **2**, 342–346.
113. Mesri EA, Cesarman E, Arvanitakes L, *et al*. Human herpesvirus-8/Kaposi's sarcoma-acssociated herpesvirus is a new transmissible virus that infects B cells. *J Exp Med* 1996; **183**, 2385–2390.