mucosal bacterial interface. The study by Maeda et al. [9] supports two alternative therapeutic approaches. Blocking the inflammatory immune response by using an IL-1β-receptor blocker represents a common theme in the modern treatment of Crohn’s disease. In the second approach, provided by antibiotic therapy, the absence of bacteria also protects Nod2-mutant mice from disease [9], which is consistent with beneficial effects of antibiotic therapy, at least in some stages of disease (reviewed in [16]). Probiotic bacteria might be another therapeutic option for bolstering the innate immune response [17] and influencing the interplay between the host mucosa and the luminal-adherent microbiota (reviewed in [16]).

Concluding remarks

The papers by Kobayashi et al. [8] and Maeda et al. [9] provide new insights into the molecular details of how this mutation leads to inflammation in Crohn’s disease and will hopefully help to open more therapeutic avenues in the future. In our opinion, the lack of mucosal defensins [7,8,10] and increased cytokine response [9,11] represent the sequential pathogenetic mechanism of the disease and are not mutually exclusive. Nevertheless, a caveat is necessary: Crohn’s disease in the mouse has not yet been observed, whatever genetic or toxic manipulations were used experimentally. Conversely, in these two pivotal studies, the ironic question to a clinician, “that is very nice but how does it apply to the mouse?” has been asked and answered to the potential and ultimate benefit of Crohn’s disease patients. And finally, Burrell Crohn’s early insights were both accurate and more prophetic than we realized.

References


Linking Kaposi virus to cancer-associated cytokines

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Viruses have evolved elaborate strategies to regulate host gene expression, thereby adapting to host stress responses against infection. In a recent report, it was shown that a human oncogenic herpesvirus, Kaposi sarcoma herpesvirus, activates the p38–MK2 pathway to stabilise cytokine transcripts. Specifically, a viral latent protein, kaposin B, binds to and activates MK2, leading to the stabilisation of AU-rich element (ARE)-containing mRNAs, which normally have only a short lifespan. Although the exact mechanism for p38–MK2 activation remains unclear, this study provides a new direction linking viral infection to selective mRNA turnover and cytokine biosynthesis.

Introduction

The p38 MAPK (mitogen-activated protein kinase) provides a nexus for signal transduction and has an essential role in numerous biological processes, including apoptosis, cell
migration, proliferation and cytokine production. Therefore, this pathway is also linked to tumorigenesis. One mechanism for the post-transcriptional regulation of specific cytokines is through the p38 substrate MAPK-activated protein kinase-2 (MAPKAP-K2 or MK2). Unexpected phenotypes from MK2-knockout mice, such as an increased resistance to endotoxin shock, revealed the essential role of this kinase in the biosynthesis of endotoxin-induced cytokines such as interleukin (IL)-6, tumour necrosis factor (TNF)α and interferon (IFN)γ [1]. The mRNAs of these cytokines are rich in the bases adenine and uracil (AU-rich elements, AREs) in their 3′-untranslated regions (3′UTRs). These AREs are involved in the degradation of mRNAs. TNFα biosynthesis becomes independent of MK2 when the ARE of the gene encoding TNFα is deleted, indicating that MK2 stabilises ARE-positive transcripts by abrogating their decay [2]. In resting cells, p38 MAPK is mainly cytoplasmic. When activated by stress signals, p38 MAPK translocates to the nucleus, resulting in MK2 activation (Figure 1). Activated MK2 exposes its nuclear-export signal (NES) and is co-exported to the cytoplasm with p38, where it protects ARE-containing mRNAs from degradation [2]. McCormick and Ganem [3] now show that a Kaposi sarcoma herpesvirus (KSHV or HHV8)-encoded protein, kaposin B, activates the p38–MK2 pathway to stabilise cytokine mRNAs, providing an elegant link between KSHV infection and elevated cytokine levels (Figure 1).

KSHV and cytokines in oncogenesis

KSHV is etiologically linked to Kaposi sarcoma (KS), which is a common endothelial neoplasm in HIV-1-infected individuals [4]. KSHV is also associated with certain lymphoproliferations, including multicentric Castleman’s disease (MCD) and primary effusion lymphoma (PEL). Long before the identification of KSHV in 1994, it was shown that cytokines have an important role in KS pathogenesis. In particular, IL-1β, IL-6 and fibroblast growth factor (FGF)-2 were shown to be secreted by KS tumour cells in culture and stimulate KS tumour-cell proliferation [5]. In early KS lesions (patch stage), KSHV is only present in a fraction of tumour cells, whereas KSHV is present in nearly every tumour cell in advanced (nodular) lesions [6]. This indicates that paracrine factors are probably important in early KS development and that KSHV confers a growth advantage to the infected population. Furthermore, cytokines are expressed in tumour cells in MCD [7], in addition to PELs. Indeed, PEL cells produce high amounts of IL-6 and their proliferation is inhibited by blocking the receptors of the IL-6 signalling pathway [8].

Gene-expression microarray profiling showed that KSHV induces the expression of multiple cytokine mRNAs after the infection of endothelial cells (ECs) in vitro [9]. Array data show that the expression of numerous cytokines is induced by KSHV, including ARE-containing [e.g. IL-6 and growth-regulated oncogene 2 (GRO2)] and ARE-negative [e.g. platelet-derived growth factor (PDGF)-A and -D, transforming growth factor (TGF)-β and angiopoietin 2 (ANG2)] transcripts (Tables 1 and 2) [10]. Several viral genes are linked to cytokine release in KS lesions: after transduction in vitro, the KSHV latent nuclear antigen (LANA1) increases the expression of MK2 [11]. The lytic viral G-protein-coupled receptor (vGPCR) induces proinflammatory cytokines, such as IL-6 and IL-8, by activating NFκB [12]. The KSHV FLICE (Fas-associated death-domain-like IL-1-α-converting enzyme)-inhibitory protein (vFLIP) also activates NFκB, by interacting with IκB kinase (IKK) [13]. vFLIP also induces IL6 gene expression in a JNK- and AP1-dependent fashion [14]. vFLIP, therefore, also contributes to the cytokine-rich milieu of KS (Figure 1).

Kaposin B stabilises cytokine mRNAs by activating MK2–p38-MAPK

The new study by McCormick and Ganem [3] provides novel mechanistic insights into how a KSHV latent protein induces cytokine secretion. Kaposin B is one of the few viral genes expressed during latency, and is therefore expressed in all KSHV tumour cells. Kaposin genes are expressed from a common locus in the viral genome: kaposin A is a product of the K12 open reading frame (ORF), whereas kaposin B and C are generated from the direct repeats (DRs) upstream of K12 or from fused DR–K12 sequences [3]. Kaposin B is the predominant product from the kaposin locus [15]. Kaposin B binds to MK2 in the nucleus through its reiterated DR2 repeats, resulting in efficient phosphorylation and, thus, the activation of MK2. This leads to the export of kaposin B with MK2 from the nucleus. Activated cytoplasmic MK2 causes a selective blockage of the degradation and/or the selective stimulation of translation of ARE-containing transcripts, thereby resulting in an increased cytokine release in kaposin-B-expressing cells (Figure 1) [3]; such cytokines include IL-6 and granulocyte macrophage-colony stimulating factor (GM-CSF). The authors show that kaposin B is the only latent KSHV protein that impairs ARE-mediated mRNA decay by activating MK2 kinase activity.

The molecular mechanisms by which kaposin B activates MK2 remain largely unclear. Cells transfected with kaposin B displayed more phosphorylated p38, which is essential for MK2 activation. The blockade of p38 activity resulted in a substantial reduction of MK2 activation and cytokine release [3]. However, how p38 is activated was not demonstrated. McCormick and Ganem [3] showed that MMK6, a kinase upstream of p38, binds to the p38–MK2 complex in the presence of kaposin B. MMK6 binding might further activate p38 activity in addition to that of MK2 (Figure 1).

Further work is needed to show whether tristetraprolin (TTP), which is a zinc-finger protein that promotes the decay of ARE transcripts, is essential for kaposin B and p38-mediated prevention of cytokine mRNA degradation. TTP is phosphorylated by MK2 and subsequently becomes inactivated (Figure 1) [16]. It will also be interesting to know whether kaposin-B-induced MK2 activation is responsible for the increased expression of other (not IL-6 and TNFα) ARE-containing cytokine mRNAs after KSHV infection of ECs (Table 1). This could be tested by microarray expression profiling of kaposin-B-transduced cells. It is noticeable that not all ARE-containing cytokine mRNAs are affected by MK2 at the transcript level and the effects of MK2 might be cell-type dependent [17].
Figure 1. KSHV activates the p38–MK2 pathway and regulates mRNA turnover. KSHV vGPCR and vFLIP are known to induce IL-6 expression, whereas LANA1 upregulates MK2 in KSHV-positive B cells. Kaposin B binds to MK2, but not to p38 MAPK, through its DR2 repeats, in the nucleus of infected cells. Stress signals, such as endotoxin or TNFα stimulation, induce the activation and nuclear translocation of p38 MAPK, resulting in the phosphorylation of MK2. MK2 phosphorylation, in addition to p38 activation, is further enhanced by kaposin B. The phosphorylation of MK2 leads to the exposure of a nuclear export signal and the nuclear export of the kaposin-B–MK2 complex. Kaposin B also stimulates MK2 kinase activity to block the rapid degradation of ARE-containing transcripts, perhaps by phosphorylating cellular targets, such as tristetraprolin (TTP). Proteins translated from protected mRNAs include inflammatory and oncogenic cytokines, such as IL-6, which in turn further amplifies the MAPK signalling pathway by an autocrine or paracrine loop.

Table 1. ARE-containing cytokine mRNAs significantly regulated by KSHV infection in primary human endothelial cells

<table>
<thead>
<tr>
<th>Upregulated</th>
<th>Downregulated</th>
</tr>
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<tbody>
<tr>
<td>AREG</td>
<td>IL5</td>
</tr>
<tr>
<td>IL1B</td>
<td>IL8</td>
</tr>
<tr>
<td>IL6</td>
<td>IL11</td>
</tr>
<tr>
<td>GRO2 (CXCL2)</td>
<td>FGF2</td>
</tr>
<tr>
<td>GRO2 (CXCL2)</td>
<td>FGF6</td>
</tr>
<tr>
<td>PTGS2 (COX2)</td>
<td>PGF</td>
</tr>
<tr>
<td>PTGS2 (COX2)</td>
<td>TGF</td>
</tr>
</tbody>
</table>

*Abbreviations: AREG, amphiregulin; COX, cyclooxygenase; CXCL, C-X-C chemokine ligand; PTGS, prostaglandin-endoperoxide synthase; SDF, stromal cell-derived factor.

*IL-6 and GM-CSF were shown to be increased by kaposin B.

Table 2. ARE-negative cytokine mRNAs that are significantly upregulated by KSHV infection in primary human endothelial cells

<table>
<thead>
<tr>
<th>Upregulated</th>
<th>Downregulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANG2</td>
<td>IL19</td>
</tr>
<tr>
<td>ANGPTL2</td>
<td>IL28A</td>
</tr>
<tr>
<td>CCL23</td>
<td>KITLG</td>
</tr>
<tr>
<td>CXCL16</td>
<td>PGF</td>
</tr>
<tr>
<td>CLEC11A</td>
<td>PDGFA</td>
</tr>
<tr>
<td>FGF12</td>
<td>PDGF</td>
</tr>
<tr>
<td>FGF18</td>
<td>TNFSF4</td>
</tr>
<tr>
<td>IL16</td>
<td>TGBF1</td>
</tr>
<tr>
<td>VEGFC</td>
<td></td>
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</table>

*Microarray data from Wang et al. [10]. ARE-mRNAs were identified from the ARED 2.0 database (http://rc.kfshrc.edu.sa/ared). All gene symbols are according to the Human Genome Organization (HUGO) [http://www.gene.ucl.ac.uk/hugo/].

*Abbreviations: ANGPTL, angiopoietin-like; CLEC, C-type lectin domain family; KITLG, KIT ligand; PGF, placental growth factor; VEGF, vascular endothelial growth factor.
downregulation of GM-CSF after KSHV infection of ECs (Table 1) compared with its increase by kaposin B in HeLa cells [3] illustrates this point.

Concluding remarks
The protective effect of kaposin B on certain ARE-mRNAs illustrates a new mechanism by which KSHV, and perhaps other viruses, regulate host gene expression by controlling the stability of mRNA (Box 1). Lytic KSHV infection induces a global host-mRNA shutoff, which is mediated by the putative viral nuclease SOX (shutoff and exonuclease), a product of KSHV ORF37. Interestingly, a highly selective escape strategy is applied by certain KSHV-induced host mRNAs through an as-yet-unidentified mechanism [18]. Striking among those protected mRNAs is IL-6, the mRNA of which is protected by kaposin B-mediated MK2 activation [3]. Because kaposin B is also induced during lytic KSHV replication, it is tempting to speculate that kaposin B activates MK2 to selectively inhibit SOX-mediated host-RNA shutoff. Because activated p38–MK2 has a role in cell motility [19], kaposin B might also induce the migration of KSHV-infected cells.

In terms of KS pathogenesis, it is noteworthy that other KSHV proteins also increase cytokine release (Figure 1) and that non-ARE-containing cytokines are elevated in KS lesions, after KSHV infection of ECs in culture (Table 2) and in the sera of individuals with KS. Linking individual KSHV proteins to the expression of these cytokines will further resolve mysteries surrounding Kaposi oncogenesis.

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Box 1. Take-home messages and outstanding questions
(i) The p38-MAPK-MK2 pathway regulates the turnover of ARE-containing mRNAs.
(ii) KSHV-encoded kaposin B activates p38-MK2 activity to stabilise ARE-containing cytokine transcripts, hence increasing cytokine production and contributing to oncogenesis.
• What are the molecular mechanisms for MK2 and p38 activation when kaposin B is present?
• Which MK2 targets are essential for kaposin-B-mediated mRNA protection?
• Is kaposin-B-induced MK2 activation also responsible for the increased expression of other ARE-containing mRNAs?
• What are the viral axes responsible for the elevated non-ARE-containing cytokine mRNAs after KSHV infection?
• How do kaposin B and other viral and cellular genes interact to regulate global mRNA stability?