Reviewer's report

Title: Gene expression profile of AIDS-related Kaposi's sarcoma: role for sialoadhesin/CD169 in tumours and circulating blood cells

Authors:

Marion M Cornelissen (m.i.cornelisen@amc.uva.nl)
Dr Antoinette AC van der Kuyl (a.c.vanderkuyl@amc.uva.nl)
Remco R van den Burg (r.vandenburg@amc.uva.nl)
Fokla F Zorgdrager (f.zorgdrager@amc.uva.nl)
Carel CJM van Noesel (c.j.vannoesel@amc.uva.nl)
Jaap J Goudsmit (j.goudsmit@crucell.com)

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Reviewer: Dr Barbara Ensoli

Level of interest: A paper whose findings are important to those with closely related research interests

Advice on publication: Unable to decide on acceptance or rejection until the authors have responded to the compulsory revisions

Reading this manuscript, I felt that it describes two different and almost independent sets of results, one relative to SAGE analysis of KS tissues as compared to normal skin, the other to the identification of CD169 as a novel marker of KS. In this context, the title of the manuscript (Gene expression profile of AIDS-related Kaposi's sarcoma: role for sialoadhesin/CD169 in tumors and circulating blood cells) remains somewhat vague due to the need to be inclusive of all results obtained.

I believe that SAGE data are interesting and may contribute significantly to the understanding of KS pathogenesis. However, I feel that these data should be described/discussed in a more organized way. In particular, the authors state that it was possible to identify three KS-specific clusters of gene expression, namely genes involved in matrix remodelling, angiogenesis and inflammation/immune responses. These three categories of gene expression reflect, indeed, what is already known of KS pathogenesis. The study promises to shed further light on KS as many additional genes involved in these processes have now been identified by the authors. However, the data relative to SAGE tags have not been organized into these three general categories. In this context, I think that the description of SAGE tags appears quite fragmentary and anecdotal (i.e. tags are not grouped and data are not presented to try to answer key questions related to KS pathogenesis). For example, the authors may give some clue to the specific role and interaction of groups of genes involved in matrix remodelling, angiogenesis, or tumor microenvironment. Similarly, they may group all specific SAGE genes involved in immune responses (including recruitment of T cells and monocyte/macrophages) and discuss the potential effects of their overexpression or downregulation on local immune activation and immune evasion processes (see, for example, Ensolì et al, Adv Canc Res 2001).

The criteria used to select specific genes for confirmatory analysis are unclear. At page 10 the authors state: "Because cells with characteristics of endothelial macrophages are present in blood and lesions of AIDS-KS patients, we focused on transcripts of adhesion molecules, secreted proteins or molecules displayed on the cell surface". These included psoriasin, A18, IL-1 R, Tie 1, Dual spec phos 1, and CD169. However, the link between KS endothelial macrophages-like cells...
and these genes is not explained. In the second part of the work, the authors proved that CD169-expressing monocyte/macrophages are present in the circulation and suggest that they may be recruited in KS lesions and tissues, explaining the multifocal nature of KS (as, indeed, previously suggested for HHV8 infected and uninfected monocytes/macrophages and endothelial macrophages (Blasig et al., J Virol, 1997; Sirianni et al, Lancet, 1997; Sirianni et al, Blood, 1998; Monini et al., Blood, 1999; reviewed in Ensoli et al, Adv Canc Res, 2001)). I think that this section of the study stands a part and it does not require the analytical presentation of all other SAGE tags identified.

Discretionary revisions

a) The authors may consider to publish SAGE analysis and data relative to upregulation of CD169 in tissues and PBMC separately. In this context, SAGE data may be presented in a more complete way, including also genes whose expression is downregulated in KS as compared to normal skin; and in a more organized way, grouping genes according to functional criteria. Data relative to CD169 would be greatly improved with analysis of risk patients or longitudinal data.

a) Material and Methods and Fig 1. Please, specify whether the 2 KS biopsies were from nodular lesions. This may explain the low expression of CD3. In fact, inflammatory cells infiltrating KS lesions are known to be more prominent in early/plaque lesions as compared to advanced-nodular lesions (see for example Ensoli et al., Adv Canc Res 2001).

b) Pag 9. The paragraph starting "In an effort to identify molecular markers for AIDS-KS......" refers to the combination of SAGE libraries and is introductory to the results described at page 10 ("Differentially expressed tags between AIDS-KS and normal skin"). This paragraph may accordingly be moved to this Results section.

c) The nucleotide sequence of RT-PCR primers should be reported in order to allow reproduction of data.

d) Pag 15. "HHV8 is known to replicate in monocyte/macrophages found in KS lesions [5,45]". However, ref [8] from Blasig et al is the pertinent work showing productive replication of HHV8 in macrophages present in tissues or docking KS lesions.

Compulsory revisions

b) Abstract: the authors state that the study is aimed at finding markers "to monitor patients at risk (of KS)"; Results: (pag 10) "Our main interest was to find transcripts that could be used as novel prognostic markers for the development of AIDS-KS, preferably measurable in blood"; (pag 12) "To evaluate a possible prognostic value of the identified transcripts, we investigated their expression in PBMC from AIDS-KS patients". However, SAGE analysis and expression of CD169 and Tie 1 were performed on pathological tissue or PBMC from AIDS-KS patients but not in risk patients without KS and no longitudinal data are provided. The authors should rephrase the text as the design of the study can only identify markers associated with KS but cannot determine their prognostic or predictive value. Alternatively, the authors should provide data relative to risk groups, or longitudinal data. In fact, further studies are required to determine the possible predictive value of the markers identified, as the author state at the end of Discussion.

c) Pag 10. The authors indicate that KS differentially expressed genes can be grouped in 3 clusters, namely "matrix remodelling genes (including matrix proteases and collagens), angiogenesis-associated genes (including secreted and membrane proteins) and immuneresponses genes". However, only specific genes but not clusters of SAGE genes were conceptually examined
for their potential effects in the histogenesis of KS lesions. Table 3 is a list of genes that is not categorized in functional groups. Data and tables relative to SAGE analysis should be accordingly organized in clusters and the relevance of such a clustered gene expression discussed in terms of KS pathogenesis. Alternatively, reference to these clusters should be deleted.

d) Pag 12: "Tie 1 is an endothelial tyrosine kinase receptor involved in angiogenesis, and as such not expected to be present at high levels in PBMC, although endothelial cells can be found in the circulation of AIDS-KS patients[29]. Please consider that, as reported in ref 29, blood from KS patients or risk individuals contains circulating cells that upon culture resemble KS cells/endothelial macrophages and not endothelial cells. In fact, after culture, KS circulating cells express VE-cadherin but not other typical endothelial cell markers (i.e. CD34, PaE) (Browning et al., Blood, 1994; Uccini et al., Am J Pathol., 1997; Monini et al., Blood, 1999; reviewed in Ensoli and Sturzl, 1998; Ensoli et al, Adv Canc Res, 2001).

e) Legend to Fig 1. "lymphocyte subpopulations and monocyte/macrophages were stained with mAbs to CD3". Please correct: monocyte/macrophages do not express CD3.

f) Fig 3: asterisks indicating statistical significance is missing.

g) Table 3. Some of the genes presented in table are indicated by an asterisk but it is unclear what the asterisk indicates.

**Competing interests:**

None declared.