The bloodsucking arthropod bite as possible cofactor in the transmission of human herpesvirus-8 infection and in the expression of Kaposi's sarcoma disease*

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Abstract. Based on a review of the literature on human herpesvirus-8 (HHV8) and Kaposi’s sarcoma (KS), and on the distribution of KS in Italy (Veneto region particularly), we hypothesize that the bite of bloodsucking arthropods is a cofactor in the seroconversion to HHV8 positivity and probably in the pathogenesis of KS. The bloodsucking arthropod releases with saliva powerful antihaemostatics and immunomodulators which may favour the replication and the establishment of the pathogen. Transmission would depend on the close contact of the child with a seropositive mother (or relatives) whose infective saliva is used to relieve itching and scratching at the arthropod bite’s sites. During any deregulation of the immune system (e.g. ageing), local immune responses to new insect bites may induce virus activation which could prelude KS insurgence. The pathogen is not directly transmitted by the arthropod which merely prepares the cutaneous microenvironment for the virus. We have therefore introduced a new category of medically important arthropods, “promoter arthropods”, besides those already defined as biological or mechanical vectors. Promoter arthropods are species able to induce in the host long-lasting, immediate or delayed-type hypersensitivity responses as well as local immunosuppression due to substances injected with their saliva. The striking variability of ORF-K1 gene of HHV8 could be due to the adaptation of the virus to the specific microenvironments resulting from the immune response to the salivary antigens characteristic of the bloodsucking arthropod species prevalent in each geographical area. It is worth noting that other viruses (especially Hepatitis B Virus) may exploit the same non-sexual transmission route.

Key words: human herpesvirus-8, Kaposi’s sarcoma, haematophagous arthropods, mosquitoes, blackflies, sandflies, biting midges, epidemiology.

Kaposi’s sarcoma (KS) is a cytokine-driven disease of multifactorial pathogenesis that starts in and remains confined to the skin, but may occasionally involve lymph nodes and visceral sites. The aetiology of the disease has been recently clarified by its close association with the infection by human herpesvirus-8 (HHV8), recently identified from HIV-related KS patients (Chang et al., 1994). Viral DNA sequences are detectable within the lesions and peripheral blood mononuclear cells of all epidemiological forms of KS (AIDS, classic, iatrogenic/post-transplantation, and endemic/African: Ambrozak et al., 1995; Dupin et al., 1995; Huang et al., 1995; Moore et al., 1995) and in two very rare lymphoproliferative diseases, named primary effusion lymphoma (Nador et al., 1996) and multicentric Castleman’s disease (Soulier et al., 1995). The aetiology of KS stresses the role of HHV8, the link to an altered immune response and the promotion/initiation by inflammatory cytokines and growth factors (Ensoli et al., 2001). Outside the context of HIV infection, however, the cofactors that are involved in disease pathogenesis remain more enigmatic (for a review, see Iscovitch et al., 2000). The transition modalities from being infected by HHV8, to having clinical manifestation of KS are still incompletely understood.

An early phase of immunostimulation and a subsequent phase of immunosuppression are risk factors (Ensoli et al., 2001). KS starts as an inflammatory process initiated by Th1-type cytokines; indices of immune activation, interferon-induced products such as neopterin and beta2-microglobulin, are elevated in classic KS (Touloumi et al., 1999). In the development of KS lesions, there is a recruitment phase in KS-prone tissues of HHV8-infected cells, which may reactivate to provide virus that, in turn, can infect surrounding cells. Then, viral gene
expression induces proliferation and angiogenesis, producing the full-grown KS lesion (Ensolii et al., 1989; Schulz, 1998; Offermann, 1999; Ensolii et al., 2001). KS initiation, therefore, is the result of a complex interaction between HHV8, the infected host and as-yet-unknown cofactors. KS is not uniformly distributed in human populations, depending on local levels of infection with HHV8. The proportion of adults with anti-HHV8 antibodies is highly variable: from 1-5% in northern Europe to about 10-40% in southern Europe, and up to >70% in sub-Saharan Africa (Gao et al., 1996). HHV8 transmission seems to occur in endemic areas according to a family pattern and with unusually low horizontal infectivity (Angeloni et al., 1998; Plancoulaine et al., 2000). The sexual route of transmission may explain only a minority of cases mostly recorded among homosexuals. The non-sexual transmission route is predominant and the passage of the virus occurs primarily from mother to child (less than 10 years of age) or among siblings during infancy, rather than between spouses or father and child (Plancoulaine et al., 2000), but the modalities of transmission remain obscure.

The human genetic component and the viral genetic variability

HHV8 infection has a peculiar ethnic/geographic pattern involving populations at higher risk to develop KS, like those of Jewish descent or living in the Mediterranean basin (i.e. Israel, Greece, Spain and Italy) (Whitty et al., 1998; Cook et al., 1999; Gambus et al., 2001). In the search for a genetic component, considering the apparent racial predisposition and geographic clustering of non-AIDS KS, several studies have focused on significantly increased (DR5) and/or decreased (DR3) HLA determinants. Results, however, are conflicting. No significant association between HLA antigens and KS was found in endemic/African KS (Melbye et al., 1987), and classic KS from Israel/Jews (Strichman-Almashanu et al., 1995), whereas a significant increase in HLA-DR5 was observed in Sardinian (Contu et al., 1984) and Greek patients, as well as in other patients of Mediterranean origin (Kaloterakis et al., 1995). HHV8 seroprevalence is higher in those communities that are to a large extent reproductively isolated from neighbouring populations (Biggar et al., 2000). Under conditions favouring inbreeding, the gene pool is less polymorphic, therefore HHV8 might be more readily and easily transmitted since the new host is genetically similar to the virus donor. Malaria pressure may have selected for genes that increase the susceptibility/resistance to HHV8 infection but the main issue remains that no clear association was recorded between human genetic traits and KS expression.

Concerning the genetic variability of HHV8, this is an ancient gamma-herpesvirus well-adapted to the human host and encoding in its genome several viral homologues of cellular genes coding for cytokines/chemokines or other proteins interfering with immunity and cell growth (Schulz, 1998). Genotypic analyses of ORF-K1, a gene located at the extreme left-hand side of the viral genome, revealed high-level variability, which defines major viral subtypes and several distinct variants or clades correlating with human population migrations and ethnic history. ORF-K1 encodes a hypervariable transmembrane protein related to the immunoglobulin receptor family containing a short-amino-acid block with high identity to the variable domains of certain immunoglobulin lambda light chains. It has been postulated that the unusually high diversity of ORF-K1 (changes are not random) reflects some unknown powerful positive biological selection pressures acting on this early lytic signalling protein (Zong et al., 1999).

Bloodsucking arthropod bites as possible cofactor in HHV8/KS transmission

The analysis of the literature on HHV8 transmission and KS expression (Whitty et al., 1998) points to various environmental risk cofactors (McHardy et al., 1984; Ziegler et al., 1997; Iscovitch et al., 2000) such as: (i) birthplace and residence in areas previously endemic for malaria (Geddes et al., 1995); (ii) the contact with volcanic soil (Ziegler, 1993; Montella et al., 1996, 2000); (iii) birth in proximity of 2 or more rivers (Ascoli et al., 2001); (iv) the amount of time spent working in proximity of water (Ziegler et al., 1997); and (v) the possession of cows (Ziegler et al., 1997). Several questions remain unanswered, including why HHV8 infection occurs in clumped foci, with little or no horizontal transmission, which are the non-sexual transmission routes, the modalities of primary HHV8 infection and of KS expression, and what is the significance of the environmental risk cofactors mentioned above.

In previous papers we have confirmed (Ascoli et al., 2002a and b) that in Italy there is overlap between sites where KS is more frequent and those where malaria was previously endemic. Malaria, however, has been eradicated from Europe more than 50 years ago (more than 75 years ago from most municipalities, particularly in northern Italy) whereas the incidence rates of classic KS have not dropped since then. A previous history of clinical malaria does not confer a higher risk of developing KS (McHardy et al., 1984; Cottoni et al., 1997). The association between malaria and HHV8 is inconsistent in Africa: in the Rift Valley area and in the East African plateau, where endemicity for malaria is generally low, KS is very frequent, whereas it is apparently rare in West Africa where the highest malaria incubation rates are recorded (Coluzzi, 1999). Finally, the view that malaria is a general risk cofactor can be challenged by the observation that HHV8 and KS are present in cold climates (Hjalgrim et al., 1998) where malaria vectors are absent, as it is the case in the Faroe Islands.

Further evidences of the correlation between KS...
incidence rates and past malarial areas were obtained from data of the Veneto Cancer Registry, and of Mantua (Ascoli et al., 2001; Ascoli et al., 2002 b). These two studies have compared the incidence rates of classic KS, endemicity for malaria in the past, and the density of blood-sucking insects. In Veneto, three different ecological zones were compared: (i) coastal cities which were endemic for malaria and are characterized by high biting rates of culicine mosquitoes and ceratopogonid midges; (ii) two internal areas with no history of malaria and lower biting rates; and (iii) mountain villages in the Alps (Cadore/Agordino). Analogously, two areas were compared in the province of Mantua: the northwestern towns of Asola/Guidizzolo (alto mantovano, soft rolling hills and fertile valleys interspersed with small lakes and springs) and the southwestern Viadensese (basso mantovano, a flat territory lying within the confluence triangle of the Po and Oglio rivers). The past distribution of malaria and the high biting rates of culicines and ceratopogonids correlate with higher incidence rates of classic KS both at a macro- and microgeographic level, with the exception of Cadore/Agordino area which however is characterized by important simulid biting rates (Rivosecchi and Coluzzi, 1962; Rivosecchi, 1986), as it is the case in the Faroe Islands.

The relationship between KS and ‘belonging to certain geographic areas’ with the above mentioned characteristics is more evident if the birthplace of KS cases is taken into account. Our microepidemiological survey confirmed the importance of birth in malarial areas and/or high-density of blood-feeding Diptera as risks for developing classic KS (Gei et al., 1995). Birthplace of most KS cases corresponds to municipalities well-known to be endemic for malaria in the past and with serious infestations of Aedes caspius (Venice) and of Simulidae (Cadore/Agordino), whereas no KS cases were recorded in malaria-free towns (Verona, Vicenza, and Rovigo) characterized by moderate to low biting rates due mainly to Culex pipiens.

Also the fact that the significant correlation between KS and malaria shown in Southern Europe becomes inconsistent in Africa suggests that the factor involved is not malaria as a disease but possibly the ecology of its vector. Thus, the main Afrotropical malaria vector mainly develops in man-made breeding sites, constituted by small temporary pools depending on rains, cannot be referred to any specific macro-habitat whereas the Mediterranean malaria vector Anopheles labranchiae is typically associated with more stable and complex aquatic biocenoses in low-land humid soils mostly characterized by quaternary sediments that frequently originate swamp areas. Such marshlands are not only the habitat of anopheline mosquitoes but also of other bloodsucking insects such as culicines of the genera Aedes, Ochlerotatus, and Coquilletidia, and biting midges (Ceratopogonidae) of the genera Leptocoriscus and Culicoides.

The correlation between African KS and volcanic soil (Ziegler, 1993), although it was not confirmed for classic KS in Sardinia (Montesu et al., 1995), was considered sound for the volcanic areas of Vesuvio in Campania (Montella et al., 1996), where in elevated seroprevalence for HHV8 is also observed (Montella et al., 2000). Thus, the impression that volcanic soils represent an environmental risk factor remains somewhat convincing. In terms of the bloodsucking arthropod hypothesis, individuals living in volcanic areas are specifically exposed to bites from sandflies (Phlebotomus spp.) which are abundant on this type of soil.

Moreover, our hypothesis is corroborated by other studies carried out prior to the HHV8 discovery that have documented: (a) frequent bites of tabanids, tsetse flies, and simulids reported by patients with KS (McHardy et al., 1984); (b) case reports of KS on the spot of insect bites (Andersson et al., 1988; Potouridou et al., 1997); (c) higher risk of developing KS in "outdoors workers", such as farmers (Cottoni et al., 1997), rice weeders or mondine (Vineis et al., 1987) or in residents of rural districts of the Po valley, a typical low-land with humid soil deriving from quaternary sediments and prone to form swamps. Finally, the 1:1 gender ratio at HHV8 seroconversion and the predominance of males developing the disease suggest a role of environmental cofactors associated with the greater mobility and outdoor activities of men, particularly in the elderly, involving a higher probability to receive bites from exophilic and exophagic bloodsucking insects.

Mechanisms of transmission: the promoter arthropod hypothesis

The evidences described above show that the environmental risk factors emerging from the analysis of the literature can be in fact condensed to a more consistent single condition involving the presence of bloodsucking arthropods mainly outdoor biting. Haematophagous arthropods can be involved in virus transmission as biological or mechanical vectors; in the first case the pathogen is adapted primarily to the arthropod, where it can replicate to invade target organs. The vertebrate host amplifies the spread of the virus. In the second case the transmission generally occurs during interrupted blood meals by viral contamination of the arthropod mouth parts. This latter condition could in principle apply sporadically to HHV8 transmission (Fischer et al., 1974) only in the presence of high densities of endophilic and anthropophilic vector species. If the hypothesis presented in this paper is correct, HHV8 seroconversion would basically depend from close contact of the child with seropositive parents or sibs, and we should introduce a third category of arthropod association with pathogen transmission with the new designation of "promoter arthropod". Such designation emphasises that the pathogen is not transmitted with the arthropod bites: the biting activity is only preparing the cellular and biochemi-
cal skin environment to the pathogen which is introduced presumably by direct contamination with infective saliva utilized to relieve itching and scratching at the arthropod bite's sites.

Indeed, salivary secretions of hematophagous arthropods contain several factors, mainly antihemostatics (blood-clotting inhibitors, vasodilators, antitrypsin) and immunomodulators, that are released with the saliva during blood-feeding and are needed to counteract the host hemostatic and inflammatory/immune responses (Lehane, 1991; Ribeiro, 1995; Gillespie et al., 2000; Kamhawi, 2000; Schoeler and Wikel, 2001). An increasingly clear concept is that the immunomodulatory properties of blood-feeding arthropods saliva can enhance pathogen transmission. This was first shown for the transmission of the Leishmania parasite by sandflies (Titus and Ribeiro, 1988). Co-injection in mice of the parasite and sand fly salivary extracts (0.1 salivary gland equivalent) increases by 5-10 fold the size of the cutaneous lesions and by 5000 times the number of parasites in the lesion. This is true for different Leishmania and sand fly species and the effect appears to last for several days, even when the parasites are injected 4 days after the salivary gland lysate (reviewed in Kamhawi, 2000). This effect, although with some differences between the New World sand fly Lutzomyia longipalpis and the Old World sand fly Phlebotomus papatasii, appears to be mediated by down-regulation of Th1-type response (inhibition of IFNγ, IL-12, NO and TNFα production) and up-regulation of Th2 response (IL-4, IL-6, IL-10, PGE₂) (Mbou, 1998; Kamhawi et al., 2000). A similar enhancing effect of salivary secretions has been described for the transmission and establishment of different viruses by mosquitoes (Edwards et al., 1998; Limesand et al., 2000) and ticks (Jones et al., 1989). Moreover, as far as we know, also in other insect/arthropod species the immunomodulatory effect of saliva seems to follow the same general trend with suppression of Th1 and induction of Th2 cytokines (Schoeler and Wikel, 2001).

The possibility of a direct link between KS and blood-feeding arthropods is supported by the pharmacological repertoire present in the saliva of hematophagous insects (Schoeler and Wikel, 2001). As HHV8 is avid in cytokines (Enso et al., 2001), viral transmission could be facilitated by a strong inflammatory reaction in response to the insect bite. KS has long been associated with the Koebner phenomenon (localisation of skin disease in the site of trauma in a host who is susceptible to that disease): for example, at sites of trauma (Berkowitz et al., 1998), surgical scars (Micali et al., 1992), skin graft (Poutourdou et al., 1997), post-irradiation (De Pasquale et al., 1999), and also insect bites (Andersson et al., 1988).

There is a clear-cut example of a link between mosquitoes, herpesvirus, and human disease. This is a form of chronic active infection with Epstein-Barr virus (EBV), which is a gamma herpesvirus like HHV8, and mosquito allergy, also known as hypersensitivity to mosquito bites. This is a severe disease that conceals clonal lymphoproliferations of EBV-infected lymphocytes (Kawa et al., 2001; Kimura et al., 2001). We should emphasize, in this context, that salivary gland extracts from blood-feeding arthropods are also strongly immunogenic and can elicit intense immune reactions. The immune response mounted by the host can follow various stages (from initial no response, to delayed- and immediate-type immune response, to no response/immunity) and may depend on several factors such as the species-specific antigens introduced with the saliva, the host genetic background and nutritional state, the exposure history to bites, the feeding modality (pool feeders vs vessel feeders), etc.

Promoter arthropods are species able to induce in the host long-lasting, immediate or delayed-type hypersensitivity responses as well as local immunosuppression due to substances injected with their saliva. This type of inflammatory reaction is attributable to several bloodsucking arthropods not necessarily associated with humans in a stable or recurrent manner, such as species of sandflies (Phlebotomus spp.), blackflies (Simulium spp.), biting midges (Culicoides spp., Leptoconops spp.), horse flies (Haematopota spp. and Chrysops spp.) and culicine mosquitoes (i.e., Ochlerotatus, Coquilletidia, and Aedes). The available epidemiological data point to the involvement of outdoor biting species and do not support any role of the common and ubiquitous domestic mosquito Culex pipiens nor of its tropical vicariant Cx quinquefasciatus, presumably because their bites seldom induce long-lasting inflammatory responses. It is also unlikely that the Afrotropical malaria vectors (Anopheles gambiae and An. funestus), or bedbugs, or lice play any role.

About the relationships of the promoter arthropod with the parasite, a well known example comes again from the sand fly P. papatasii, whose bites can induce in humans strong hypersensitivity reactions (Belkaid et al., 2000). In mice pre-exposed to sand fly bites there is a 2 to 5-fold increase of infiltrating cells (neutrophils, eosinophils, macrophages, dendritic cells, lymphocytes) and approximately a 10-fold induction of IFNγ and IL-12 in comparison to naive mice. This response induced by previous exposure to uninfected sandfly bites can confer protection against cutaneous leishmaniasis by activation of infected macrophages and promotion of a Th1-type response (Kamhawi et al., 2000). Thus, it is tempting to speculate that insect bites in skin KS prone areas (extremities, nose, ears) might provide the needed inflammatory cytokines rich milieu to HHV8-infected circulating KS-progenitors (lymphocytes, monocytes and spindle-like cells). This might represent a major stimulus for HHV8 reactivation favouring the latent-to-lytic infection switch with angiogenesis, inflammatory infiltration and spindle-cell induction. Under this hypothesis, living in zones infested with hematophagous insects could promote not only HHV8 infections but also KS development.
Concluding remarks and suggested research protocols

We can summarize the essential argument of our working hypothesis as follows: HHV8 is not transmitted efficiently without the concurrent biting activity of an haematophagous arthropod. The close contact with the virus carriers would be a necessary-but-not-sufficient condition for effective transmission. Entry and establishment of the virus in uninfected individuals may be favoured by the local immunomodulation induced by the arthropod saliva and will be essentially dependent on human behaviour, i.e. the use of saliva at the itching and scratching sites. The tissue injury determined by the bite could offer a suitable entry point. Moreover, the itching and the subsequent scratching could increase the chances of infection. After establishment into a favorable micro-environment the virus could escape the mounting immune response by intra-cellular latency and eventually be reactivated, sometimes much later during life, as a consequence of an immune activation and a Th1-type cytokine production in a more general context of immunodepression.

Substantial progress in testing the hypothesis of the promoter arthropod could come from an experimental laboratory model utilizing monkeys. This model, however, is as yet unavailing, and experimental field protocols on human populations appear at the moment more promising in spite of the difficulties that are often experienced in controlling confounding factors. Much can be learned by more detailed epidemiological studies in Sardinia and Veneto regions of Italy (Cottoni et al., 1997; Calabrò et al., 1998). In Sardinia the reanalysis of the registered cases in terms of residence and birthplace can be very informative particularly if the data are complemented by ad hoc updated entomological surveys. The Veneto study is going to provide the opportunity for a comparative analysis of HHV8 strains in Venice and Cadore where the infection is expected to be associated with different promoter insects, i.e. culicine mosquitoes or ceratopogonid midges in Venice vs simulids in Cadore. If the viral material from these two areas will be found differentiated for the ORF-K1 gene or for the VR loop, this finding would support the hypothesis of the unusual genetic variability of HHV8, somehow connected to the virus adaptation to specific cellular-biochemical environments produced by the immune response to salivary antigens characteristic of the biting species, more common in certain geographic areas. Thus, the promoter arthropods would just represent the different biological selection pressure hypothesized by Hayward (1998) and Zong et al. (1999). Feasible research protocols aiming at testing the hypothesis of the involvement of insect bites in HHV8 transmission in populations from endemic areas (sub-Saharan Africa) are listed below.

(i) Socio-anthropological evaluation of the use of saliva in relation to insect bites. This will consist in the preparation and administration of a consent questionnaire with the aim to evaluate to what extent saliva is used by parents and relatives to heal insect bites on children. The questionnaire could be administered to children of the first three classes of primary schools (5-9-years old), focusing on the following questions: (a) How many blood-sucking insects do you know? (b) Have you ever been bitten by one of them? (c) If yes, what kind of insect? (d) Were you bitten inside or outside the house? (e) In what period of the year: rainy season or dry season? (if applicable). (f) In what period of the day: morning, afternoon, evening, night? (g) What was the skin reaction: pain, itching, pimple? (h) What was the duration of the reaction: <3 days, from 3 to 7 days, >7 days? (i) What kind of treatment did you receive: none, ointment, saliva, other? (j) If saliva was used, explain the way it was applied: sucking, lapping, transfer of saliva by hand? From preliminary information, this study applied in various sub-Saharan countries, in various regions of Italy and Central Europe is revealing a south-to-north negative cline in the frequency of use of saliva.

(ii) Trials on Burkinabe children born and resident in Italy. The Association of Burkinabes that migrated to Lombardia (Northern Italy) has kindly agreed to have their children and parents tested for HHV8 seropositivity. With the reasonable assumption that these children were subject to lower biting rates from hematophagous insects, as compared to similar cohorts born and resident in Burkina Faso, a comparative analysis on HHV8 seropositivity will be carried out. For each child included in the Italian trial two children of the same age and social status will be recruited in Burkina Faso and tested by the same method.

(iii) Multicentric trials to evaluate the importance of insect bites in HHV8 transmission. Based on similar protocols should consist in the recruitment of about 500 children (3 to 5-years-old) per trial chosen among those seronegative with seropositive mother and relatives. The seroconversion will be monitored every six months with serological tests and PCR on the child’s saliva. Each cohort will be subdivided in two subsamples, one of which (100 children) left as control, and the other (200 children) protected from insect bites with all available tools (residual insecticides indoor spraying, insecticide-treated bednets, and repellents). Furthermore, the latter cohort could be further divided in two subgroups, one of which will be the target of a health education intervention on mothers and relatives whose awareness on the risk of the use of saliva to cure insect bites is raised.

The non-sexual route of transmission which is hypothesized in this paper to be the main transmission route for HHV8 could be also important for other herpesviruses such as Epstein-Barr virus (EBV), and it could be at least a secondary transmission mechanism for the Hepatitis B Virus (HBV).
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References


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