Parassitologia 44: 123-129, 2002

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

M. Coluzzi[1], D. Manno[1, 2], S. Guzzinati[3], S. Tognazzo[3], P. Zambon[4], B. Arcà[1], C. Costantini[1], V. Ascoli[2]

[1] *Sezione di Parassitologia, Dipartimento di Scienze di Sanità Pubblica, Centro Collaboratore Organizzazione Mondiale della Sanità, Università "La Sapienza" di Roma, Italy;* [2] *Anatomia Patologica, Dipartimento di Medicina Sperimentale e Patologia, Università "La Sapienza" di Roma, Italy;* [3] *Servizio di Epidemiologia dei Tumori, Registro Tumori del Veneto, Azienda Ospedaliera di Padova, Italy;* [4] *Dipartimento di Scienze Oncologiche e Chirurgiche, Registro Tumori del Veneto, Università di Padova, Italy.*

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 releave 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-trasplantation, and endemic/African: Ambroziak *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 Iscovich *et al.*, 2000). The transition modalities from being infected by HHV8, to having clinical manifestation of KS are still uncompletely 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 beta$_2$-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

* *The paper is dedicated to the memory of Professor Ettore Biocca. We feel that a multidiscplinary-minded scientist as he was would appreciate this attempt to establish links between the parasite and the arthropod via human behaviour, i.e. links between virology, medical entomology and anthropology.*

Correspondence: Professor Valeria Ascoli, Anatomia Patologica, Dipartimento di Medicina Sperimentale e Patologia, Università di Roma "La Sapienza", Viale Regina Elena 324, 00161 Roma, Italy, tel +39 06 44703550, e-mail: valeria.ascoli@uniroma1.it

expression induces proliferation and angiogenesis, producing the full-grown KS lesion (Ensoli *et al.*, 1989; Schulz, 1998; Offermann, 1999; Ensoli *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) (Whitby *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 (Whitby *et al.*, 1998) points to various environmental risk cofactors (McHardy *et al.*, 1984; Ziegler *et al.*, 1997; Iscovich *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 plateaux, where endemicity for malaria is generally low, KS is very frequent, whereas it is apparently rare in West Africa where the highest malaria inoculation 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 malarious 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 Viadanese (*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 cerapogonids 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 malarious areas and/or high-density of blood-feeding Diptera as risks for developing classic KS (Geddes *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 Simuliidae (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 refereed 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 *Leptoconops* 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), wherein 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 exophylic 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 releave itching and scratching at the arthropod bite's sites.

Indeed, salivary secretions of hematophagous arthropods contain several factors, mainly antihemostatics (blood-clotting inhibitors, vasodilators, antiplatelet) 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 papatasi*, 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, PGE_2) (Mbow *et al.*, 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 haematophagous insects (Schoeler and Wikel, 2001). As HHV8 is avid in cytokines (Ensoli *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 (Potouridou *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 mosquitos, 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. papatasi*, 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 naïve 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 haematophagous 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 unavailable, 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, hitching, 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 300 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 hyopothesized 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).

Acknowledgements

Entomological data on Italian bloodsucking arthropods were verified and discussed by one of us (MC) with the following colleagues: Alberto Baseggio, Leo Rivosecchi and Renato Zamburlini for Veneto region; Luca Rossi for Biella area; Carlo Contini for Sardinia region. We express our appreciation to the Director of the Natural History Museum of the Faroe Islands, Dr Dorothy Bloch for kindly confirming the presence of simulid infestation. The 'promoter arthropod' hypothesis was discussed with the following colleagues: Geneviève Milon (Institut Pasteur, Paris, France), Didier Fontanille (IRD, Montepellier, France), Robin Weiss (University College, London, UK), José Ribeiro (NIH, Bethesda, USA), Ogobara Doumbo (Bamako Medical School, Mali). We are also grateful to Michele La Placa (Bologna), Luisella Calabrò (Padova) and the colleagues of the Faculty of Medicine of the University of Rome "La Sapienza": Nicola Orsi, Ferdinando Dianzani, Antonio Angeloni and Alberto Faggioni for useful information and encouragement. In view of the lack of speficic funds to this research, part of the expenses were covered by malaria research grants from WHO/TDR, Compagnia di San Paolo, and MUIR.

References

Ambroziak JA, Blackbourn DJ, Herndier BG, Glogau RG, Gullett JH, McDonald AR, Lennette ET, Levy JA (1995). Herpes-like sequences in HIV-infected and uninfected Kaposi's sarcoma patients. Science 268: 582-583.

Andersson L, Heyden G, Krekmanov L (1988). Insect bite associated Kaposi's sarcoma with oral lesions. Int J Oral Maxillofac Surg 17 (2): 76-77.

Angeloni A, Heston L, Uccini S, Sirianni MC, Cottoni F, Masala MV, Cerimele D, Lin SF, Sun R, Rigsby M, Faggioni A, Miller G (1998). High prevalence of antibodies to human herpesvirus 8 in relatives of patients with classic Kaposi's sarcoma from Sardinia. J Infect Dis 177: 1715-1718.

Ascoli V, Belli S, Benedetti M, Trinca S, Ricci P, Comba P (2001). High incidence of classic Kaposi's sarcoma in Mantua, Po Valley, Northern Italy (1989-1998). Br J Cancer 85: 379-382.

Ascoli V, Manno D, Guzzinati S, Tognazzo S, Zambon P, Arcà B, Costantini C, Coluzzi M (2002a). La puntura di artropodi ematofagi quale possibile cofattore nella trasmissione di HHV8 (human herpes virus 8) e nell'espressione del sarcoma di Kaposi. Rend Fis Accad Lincei 13 (in press).

Ascoli V, Zambon P, Manno D, Guzzinati S, Zorzi M, Arcà B, Costantini C, Coluzzi M (2002b). Variability in the incidence of classic Kaposi's sarcoma in three areas (coast, inland, mountains) of Veneto, Northern Italy. Tumori (submitted)

Belkaid Y, Valenzuela JG, Kamhawi S, Rowton E, Sacks DL, Ribeiro JM (2000). Delayed-type hypersensitivity to *Phlebotomus papatasi* sand fly bite: an adaptive response induced by the fly? Proc Natl Acad Sci USA 97: 6704-6709.

Berkowitz KD, Bonner AC, Makimaa B, Flash JP, Sasken H, Blaise JF (1998). Trauma-induced Kaposi's sarcoma of the hallux. An unusual case. J Am Pediatr Med Assoc 88: 500-505.

Biggar RJ, Whitby D, Marshall V, Linhares AC, Black F (2000). Human herpesvirus 8 in Brazilian Amerindians: a hyperendemic population with a new subtype. J Infect Dis 181: 1562-1568.

Calabrò ML, Sheldon J, Favero A, Simpson GR, Fiore JR, Gomes E, Angarano G, Chieco-Bianchi L, Schulz TF (1998). Seroprevalence of Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8 in several regions of Italy. J Hum Virol 1: 207-213.

Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS (1994). Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 266 (5192): 1865-1869.

Coluzzi M (1999). The clay feet of the malaria giant and its African roots: hypotheses and inferences about origin, spread and control of *Plasmodium falciparum*. Parassitologia 41: 277-283.

Contu L, Cerimele D, Pintus A, Cottoni F, La Nasa G (1984). HLA and Kaposi's sarcoma in Sardinia. Tissue Antigens 23: 240-245.

Cook PM, Whitby D, Calabrò ML, Luppi M, Kakoola DN, Hjalgrim H, Ariyoshi K, Ensoli B, Davison AJ, Schulz TF (1999). Variability and evolution of Kaposi's sarcoma-associated herpesvirus in Europe and Africa. International Collaborative Group. AIDS 13: 1165-1176.

Cottoni F, Masala MV, Budroni M, Rosella M, Satta R, Locatelli F, Montesu MA, De Marco R (1997). The role of occupation and a past history of malaria in the etiology of classic Kaposi's sarcoma: a case-control study in north-east Sardinia. Br J Cancer 76: 1518-1520.

De Pasquale R, Nasca MR, Micali G (1999). Postirradiation primary Kaposi's sarcoma of the head and neck. J Am Acad Dermatol 40: 312-314.

Dupin N, Grandadam M, Calvez V, Gorin I, Aubin JT, Havard S, Lamy F, Leibowitch M, Huraux JM, Escande JP *et al* (1995). Herpesvirus-like DNA sequences in patients with Mediterranean Kaposi's sarcoma. Lancet 345 (8952): 761-762.

Edwards JF, Higgs S, Beaty BJ (1998). Mosquito feeding-induced enhancement of Cache Valley virus (Bunyaviridae) infection in mice. J Med Entomol 35: 261-265.

Ensoli B, Nakamura S, Salahuddin SZ, Biberfeld P, Larsson L, Beaver B, Wong-Staal F, Gallo RC (1989). AIDS-Kaposi's sarcoma-derived cells express cytokines with autocrine and paracrine growth effects. Science 243 (4888): 223-226.

Ensoli B, Sgadari C, Barillari G, Sirianni MC, Sturzl M, Monini P (2001). Biology of Kaposi's sarcoma. Eur J Cancer 37: 1251-1269.

Fischer RG, Falk LA, Rytter R, Burton GJ, Luecke DH, Deinhardt F (1974). Herpesvirus saimiri: viability in four species of hematophagous insects and attempted insect transmission to marmosets. J Natl Cancer Inst 52: 1477-1481.

Gao SJ, Kingsley L, Li M, Zheng W, Parravicini C, Ziegler J, Newton R, Rinaldo CR, Saah A, Phair J, Detels R, Chang Y, Moore PS (1996). KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma. Nat Med 2: 925-928.

Gambus G, Bourboulia D, Esteve A, Lahoz R, Rodriguez C, Bolao F, Sirera G, Muga R, del Romero J, Boshoff C, Whitby D, Casabona J (2001). Prevalence and distribution of HHV8 in different subpopulations, with and without HIV infection, in Spain. AIDS 15: 1167-1174.

Geddes M, Franceschi S, Balzi D, Arniani S, Gafà L, Zanetti R (1995). Birthplace and classic Kaposi's sarcoma in Italy. J Natl Cancer Inst 87: 1015-1017.

Gillespie RD, Mbow ML, Titus RG (2000). The immunomodulatory factors of bloodfeeding arthropod saliva. Parasite Immunol 22: 319-331.

Hayward GS (1999). KSHV strains: the origins and global spread of the virus. Cancer Biol 9: 187-199.

Hjalgrim H, Tulinius H, Dalberg J, Hardarson S, Frisch M, Melbye M (1998). High incidence of classical Kaposi's sarcoma in Iceland and the Faroe Islands. Br J Cancer 77: 1190-1193.

Huang YQ, Li JJ, Kaplan MH, Poiesz B, Katabira E, Zhang WC, Feiner D, Friedman-Kien AE (1995). Human herpesvirus-like nucleic acid in various forms of Kaposi's sarcoma. Lancet 345 (8952): 759-761.

Iscovich J, Boffetta P, Franceschi S, Azizi E, Sarid R (2000).

Classic Kaposi sarcoma: epidemiology and risk factors. Cancer 88: 500-517.

Jones LD, Hodgson E, Nuttall PA (1989). Enhancement of virus transmission by tick salivary glands. J Gen Virol 70: 1895-1898.

Kamhawi S (2000). The biological and immunomodulatory properties of sand fly saliva and its role in the establishment of *Leishmania* infection. Microb Infect 2: 1765-1773.

Kamhawi S, Belkaid Y, Modi G, Rowton E, Sacks D (2000). Protection against cutaneous leishmaniasis resulting from bites of uninfected sand flies. Science 290: 1351-1354.

Kaloterakis A, Papasteriades C, Filiotou A, Economidou J, Hadjiyannis S, Stratigos J (1995). HLA in familial and nonfamilial Mediterranean Kaposi's sarcoma in Greece. Tissue Antigens 45: 117-119.

Kawa K, Okamura T, Yagi K, Takeuchi M, Nakayama M, Inoue M (2001). Mosquito allergy and Epstein-Barr virus-associated T/natural killer-cell lymphoproliferative disease. Blood 98: 3173-3174.

Kimura H, Hoshino Y, Kanegane H, Tsuge I, Okamura T, Kawa K, Morishima T (2001). Clinical and virologic characteristics of chronic active Epstein-Barr virus infection. Blood 98: 280-286.

Limesand KH, Higgs S, Pearson LD, Beaty BJ (2000). Potentiation of vesicular stomatitis New Jersey virus infection in mice by mosquito saliva. Paras Imunol 22: 461-467.

Lehane MJ (1991). Biology of blood-sucking insect. Harper Collins Academic, London, UK.

Mbow ML, Bleyemberg JA, Hall LR, Titus RG (1998). *Phlebotomus papatasi* sand fly salivary gland lysate down-regulates a Th1, but up-regulates a Th2, response in mice infected with *Leishmania major*. J Immunol 161: 5571-5577.

McHardy J, Williams EH, Geser A, de The G, Beth E, Giraldo G (1984). Endemic Kaposi's sarcoma: incidence and risk factors in the West Nile District of Uganda. Int J Cancer 33: 203-212.

Melbye M, Kestens L, Biggar RJ, Schreuder GM, Gigase PL (1987). HLA studies of endemic African Kaposi's sarcoma patients and matched controls: no association with HLA-DR5. Int J Cancer 39: 182-184.

Micali G, Gasparri O, Nasca MR, Sapuppo A (1992). Kaposi's sarcoma occurring de novo in the surgical scar in a heart transplant recipient. J Am Acad Dermatol 27: 273-274.

Montella M, Franceschi S, Geddes M, Arniani S, Cocchiarella G (1996). Classic Kaposi's sarcoma and volcanic soil in southern Italy. Lancet 347 (9005): 905.

Montella M, Serraino D, Crispo A, Rezza G, Carbone S, Tamburini M (2000). Is volcanic soil a cofactor for classic Kaposi's sarcoma? Eur J Epidemiol 16: 1185-1186.

Montesu MA, De Marco R, Cottoni F (1995). Soil silicates and Kaposi's sarcoma in Sardinia. Lancet 346 (8987): 1436-1437.

Moore PS, Chang Y (1995). Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and without HIV infection. N Engl J Med 332: 1181-1185.

Nador RG, Cesarman E, Chadburn A, Dawson DB, Ansari MQ, Sald J, Knowles DM (1996). Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpesvirus. Blood 88: 645-656.

Offermann MK (1999). Consideration of host-viral interactions in the pathogenesis of Kaposi's sarcoma. J Acquir Immune Defic Syndr 21, Suppl 1: S58-65.

Plancoulaine S, Abel L, van Beveren M, Tregouet DA, Joubert M, Tortevoye P, de The G, Gessain A (2000). Human herpesvirus 8 transmission from mother to child and between siblings in an endemic population. Lancet 356 (9235): 1062-1065.

Potouridou I, Katsambas A, Pantazi V, Armenaka M, Vareltzidis A, Stratigos J (1997). Koebner phenomenon in classic Kaposi's sarcoma. Acta Derm Venereol 77: 481.

Ribeiro JM (1995). Blood-feeding arthropods: live syringes or invertebrate pharmacologists? Infect Agents Dis 4: 143-152.

Rivosecchi L, Coluzzi M (1962). Tre simulidi *Simulium aureum* Fries (s l), *Simulium erytrocephalum* DeGeer, *Simulium reptans* (L) che in Italia pungono l'uomo. Parassitologia 4: 181-190.

Rivosecchi L (1986). Contributo alla conoscenza dei Simulidi italiani. XXVII. Le specie che attaccano in massa l'uomo e gli animali domestici nell'Italia nord-orientale. Riv Parassitol III (XLVII), 1.

Schulz TF (1998). Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8). J Gen Virol 79: 1573-1591.

Schoeler GB, Wikel SK (2001). Modulation of host immunity by hematophagous arthropods. Annals Trop Med Parasitol 95: 755-771.

Soulier J, Grollet L, Oksenhendler E, Cacoub P, Cazals-Hatem D, Babinet P, d'Agay MF, Clauvel JP, Raphael M, Degos L, Sigaux F (1995). Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. Blood 86: 1276-1280.

Strichman-Almashanu L, Weltfriend S, Gideoni O, Friedman-Birnbaum R, Pollack S (1995). No significant association between HLA antigens and classic Kaposi sarcoma: molecular analysis of 49 Jewish patients. J Clin Immunol 15: 205-209.

Touloumi G, Hatzakis A, Potouridou I, Milona I, Stratigos J, Katsambas A, Giraldo G, Beth-Giraldo E, Biggar RJ, Mueller N, Trichopoulos D (1999). The role of immunosuppression and immune-activation in classic Kaposi's sarcoma. Int J Cancer 82: 817-821.

Titus RG, Ribeiro JM (1988). Salivary gland lysates from the sandflies *Lutzomyia longipalpis* enhance *Leishmania* infectivity. Science 239: 1306-1308.

Vineis P, Terracini B, Ciccone G, Cignetti A, Colombo E, Donna A, Maffi L, Pisa R, Ricci P, Zanini E *et al* (1987). Phenoxy herbicides and soft-tissue sarcomas in female rice weeders. A population-based case-referent study. Scand J Work Environ Health 13: 9-17.

Whitby D, Luppi M, Barozzi P, Boshoff P, Weiss RA, Torelli G (1998). Human herpesvirus-8 seroprevalence in blood donors and lymphoma patients from different regions of Italy. J Natl Cancer Inst 90: 395-397.

Ziegler JL (1993). Endemic Kaposi's sarcoma in Africa and local volcanic soils. Lancet 342 (8883): 1348-1351.

Ziegler JL, Newton R, Katongole-Mbidde E, Mbulataiye S, De Cock K, Wabinga H, Mugerwa J, Katabira E, Jaffe H, Parkin DM, Reeves G, Weiss R, Beral V (1997). Risk factors for Kaposi's sarcoma in HIV-positive subjects in Uganda. AIDS 11: 1619-1626.

Zong JC, Ciufo DM, Alcendor DJ, Wan X, Nicholas J, Browing PJ, Rady PL, Tyring SK, Orenstein JM, Rabkin CS, Su IJ, Powell KF, Croxson M, Foreman KE, Nickoloff BJ, Alkan S, Hayward GS (1999). High-level variability in the ORF-K1 membrane protein gene at the left end of the Kaposi's sarcoma-associated herpesvirus genome defines four major virus subtypes and multiple variants or clades in different human populations. J Virol 73: 4156-4170.