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Primary Research: Short Communication: Evidence Supporting Rare AIDS-Kaposi's Sarcoma Metastasis In Keeping With Their Vascular Endothelial Evolution

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Abstract

Background: It is postulated that the unusual manifestations of Kaposis's sarcoma cells in nonendothelial brain tissues and on eyeballs in advanced acquired immune deficiency syndrome (AIDS) cases are metastasized AIDS-Kaposi's sarcoma cells arising from vascular endothelial cells.

Methods: Experiments were performed to explore the above hypothesis by testing for intercellular adhesion molecule-1 (CD54 antigens) on cutaneous AIDS-Kaposi's sarcoma cells as well as on AIDS-Kaposi's sarcoma cells isolated from eyeballs as studies have illustrated that, unlike localized Kaposi's sarcoma cells of primary lesions, proliferating Kaposi's sarcoma cells in proximity to primary lesions express a negative or diminished phenotype when evaluated for identical surface antigens. Parallel CD54 antigen tests were done on vascular endothelial cells and monocytes/macrophages as endothelial cells are considered evolutionarily related to Kaposi's sarcoma cells and monocytes/macrophages are ideal CD54 antigen positive controls.

Results: Our data showed that only AIDS-Kaposi's sarcoma cells of the eyes did not express CD54 antigens.

Conclusions: We therefore report that our findings support the postulation suggesting AIDS-Kaposi's sarcoma dissemination in advanced AIDS patients in keeping with their vascular endothelial heredity.

Introduction

Recent evidence documenting the preponderance of endothelial cells (ECs) in and about Kaposi's sarcoma (KS) lesions is just one factor that is indicative of primary KS formation from EC defects [1,2]. Consistent with this knowledge is the postulation that the occasional appearances of Kaposi's sarcoma cells (KSCs) in nonendothelial cerebral tissues and on eyeballs in advanced acquired immune deficiency syndrome (AIDS) patients are secondary AIDS-KS aggregates, that owe their existence to migrated AIDS-KSCs [1–3]. This observation is further refined to the understanding that these seldom and, if any, disseminating AIDS-KSCs are small in population as compared to the majority of AIDS-KSCs, which remain localized, but also that there is the need to characterize the difference between these two populations of AIDS-KSCs in order to an-

alytically show that dissemination, possibly, has taken place [4].

According to an extensive histological determination, a direct correlation exists between surface ligand (Ag) expression and proliferation among KSCs [5]. The same analysis also documents a similar correlation between Ag expression and metastasis for an endothelial tumor [5]. These findings suggest that because of correlations and ancestral connections, Ag integrity on AIDS-KSCs is crucial in establishing merit of the metastasis theory particularly as Ag sensitivity is frequently utilized in histopathology not only to diagnose tumor dissemination but also to predict benign to cancerous transformation [5].

Now, when peripheral blood lymphocytes (PBLs) are allosensitized with recombinant interleukin-2 (rIL-2) they develop into cytotoxic lymphokine-activated killer (LAK) cells [6]. These LAK-cells (effectors) are capable of lysing a wide variety of tumors (targets) both in vitro and in vivo through Ag mediated interactions [6,7]. In addition, the Ag mediated interactions of LAK effectors also target and cause cytotoxicity to vascular ECs and monocytes/macrophages (MΦ) [8,9]. It was therefore proposed that the presence of heterogeneous LAK-cell Ags on vascular ECs, MΦ and tumor cells would make it possible to obviate of the complicating injury to ECs and MΦ in LAK-cell immunotherapy via the application of monoclonal antibodies (mAbs) to selectively inhibit EC and MΦ damage [10].

Pursuant to this approach, we incorporated the cold-target inhibition technique [11] in chromium release assays to detect possible heterogeneity of LAK-cell Ags on AIDS-KSCs, vascular ECs and MΦ[10]. Data accumulated then showed that unlike cutaneous AIDS-KS10 cells, AIDS-KS21 cells collected from ocular lesions did not cold-target inhibit EC lysis [10]. This suggested differences in LAK-cell Ags of AIDS-KS21 cells versus AIDS-KS10 cells and ECs, specifically, pointing to intercellular adhesion molecule-1 (ICAM-1) or CD54 Ags as studies have repeatedly elucidated ICAM-1 on targets binding to lymphocyte function associated antigen-1 (LFA-1) on LAK effectors in LAK-cell attacks [12]. In this project we explore the metastasis postulation by testing for CD54 Ag presentations on vascular ECs, MΦ and AIDS-KS21/-KS10 cells. We report that our combined data support the postulation suggesting AIDS-KS or epidemic-KS dissemination within the confines of their vascular endothelial heredity.

Results and Discussion
LAK-cell cytotoxicities to tumor cells, virally or microbially infected cells, vascular ECs and MΦ occur via the adhesion of effectors to targets facilitated by LFA-1 Ags on effectors interacting with ICAM-1 or CD54 moieties (Ags) on targets [12]. RH1-38 is a murine mAb, which inhibits

LAK-cell cytotoxicity by binding to the homologous β chains or CD18 Ags of LFA-1 [13]. In our present study we treated LAK effectors with RH1-38 in chromium release cytotoxicity assays to test for the presence of CD54 Ags on MΦ, vascular ECs and on both AIDS-KS21 and AIDS-KS10 cells. Our results herein showed that although RH1-38 did not inhibit killing of AIDS-KS21 cells, it concomitantly inhibited rupture of AIDS-KS10 cells, vascular ECs and MΦ (Table 1). Combining this finding with our previous data, where AIDS-KS21 cells failed to cold-target inhibit lysis of vascular ECs, as otherwise demonstrated by AIDS-KS10 cells [10], summarize that AIDS-KS21 cells bore either diminished or negative CD54 Ag composition. Published papers to date have reported CD54 Ags on several other AIDS-KS cell lines unaffected following multiple passages in vitro [14], and that no significant immunophenotyping differences exist between AIDS-KS3, -KS6 and -KS8 cells at comparable stages [15].

In our earlier paper, we specified that AIDS-KS21 cells originated from an eye lesion, whereas, AIDS-KS10, -KS8 and -KS3 cells were cutaneous, visceral and pleural fluid isolates, respectively [10]. Juxtaposed was put the observation that the differences in reactions of AIDS-KS21 cells may be related to location, however, a plausible explanation could not be proposed [10]. In light of recent evidence, we suspect the following histopathogenesis. Kaposi's sarcoma (KS) are widely accepted as EC derivatives of either vascular or lymphatic lineage, but the precise EC progenitor remains unestablished [12,16,17]. Nevertheless, evidence illustrating histological similarities between KSCs and ECs, coupled with EC Ag detections on KSCs, lend credence to this evolution [1,2,16,17]. KS, by itself, does not behave like other soft tissue sarcomas, which can proceed to metastasize after growing locally; it is a stationary tumor, which arises in numerous sites via multicentric development [1,2,4,16,17]. However, AIDS-KS or epidemic-KS presents an aggressive form capable of drastic histopathogenesis [1,2,4,16,17]. One manifestation of such aggressiveness is that on rare instances AIDS-KS can involve brain and, or, eyes in advanced AIDS patients [1,2]. It is widely held that since eyes and brain are devoid of lymphatic endothelium, these organs are unlikely sources of primary KS if KSCs are indeed derived from lymphatic endothelium [1,2]. Aside from this insight, research has shown AIDS-KS capable of angiogenesis, metastasis and tumorigenicity in mice [18,19]. Owing to such detail, the occurrences of AIDS-KS in brain and on eyes in propinquity to cranial nerves, minute capillaries and associated blood vessels are attributed to vascular endothelial alteration [1–3]. And in keeping with this evolutionary profile, occurrences of AIDS-KS in nonendothelial, nonvascularized cerebral sites and on conjunctiva are postulated to be products of AIDS-KS metastasis [1–3]. One investigation reported that al-

Table 1: Results of ^{51}Cr release assays of labeled (H) vascular ECs, MΦ, AIDS-KS10 and AIDS-KS21 cells all at 50:1 E:H ratio and with: NMS-1 at 1:400 and 1:100 dilutions (columns 2 & 3), mAb RH1-38 also at 1:400 and 1:100 dilutions (columns 4 & 5), neither RH1-38 nor NMS-1 (column 6). Values in brackets indicate percentage (%) inhibition of NMS-1 (1:400) lysis.

E:Ab	NMS-1 (1:400)	NMS-1 (1:100)	RH1-38 (1:400)	RH1-38 (1:400)	NoRH1-38/NMS-1
Cell Type (H)	*Experiment # 1 Percent (%) Lysis ± SEM*				
I. MΦ	60 ± 4.1	50 ± 4.9	19 ± 0.9(68)	1 ± 0.8(98)	60 ± 3.9
II. Vascular ECs	67 ± 2.8	68 ± 2.7	30 ± 0.6(55)	5 ± 0.3(93)	66 ± 1.3
III. AIDS-KS10	85 ± 0.3	75 ± 1.3	33 ± 0.7(61)	8 ± 0.6(91)	80 ± 2.4
IV. AIDS-KS21	81 ± 4.3	80 ± 2.2	75 ± 2.0 (7)	74 ± 4.5(9)	81 ± 4.9
	Experiment # 2 Percent (%) Lysis ± SEM				
I. MΦ	40 ± 1.7	39 ± 2.8	12 ± 1.4(70)	4 ± 1.0(90)	44 ± 1.0
II. Vascular ECs	80 ± 1.2	75 ± 1.5	38 ± 0.9(53)	10 ± 0.9(88)	77 ± 0.3
III. AIDS-KS10	76 ± 2.0	74 ± 1.2	30 ± 0.3(60)	9 ± 0.6(88)	75 ± 2.0
IV. AIDS-KS21	55 ± 1.2	54 ± 1.3	49 ± 0.9(11)	54 ± 2.3 (2)	52 ± 2.1

though AIDS-KS skin biopsies expressed the cell surface proenzyme, procathepsin L, neighboring AIDS-KSCs, which were characterized predominantly by vascular endothelial cell proliferation, as in an angiomatous lesion, did not express the protease, eventhough, adjoining blood vessels stained positively [20]. Another examination found diminished or negative reactions for UEA-1 lectin, HLA-DR and OKM5 Ags on KSCs, which were in adjacent proliferating areas slightly distant from their EC-mix surrounding capillaries [5]. In addition, cells of the vascular endothelial tumor, malignant angioendothelioma, which bore the blood group H Ag in premetastatic stage had somehow lost the Ag when metastasis occurred [5]. Further probing revealed lymphatic tumors not to display this differentiating property [5]. Therefore, this invasive phenomenon possibly explains the paucity of CD54 Ags on AIDS-KS21 cells that rendered them unprotected following mAb treatment. Such a course would be consistent with the view that the failure of KSCs to exhibit uniform staining for Ags suggests differences in their proliferation stages particularly as mutated cells are understood to be capable of altering their surface Ag expression [2,17].

Conclusions
In conclusion, the situation of AIDS-KSCs on eyeballs is possibly a consequence of rare epidemic-KS dissemination in keeping with their vascular endothelial evolution. The relevance of our findings could help to better understand the nature of AIDS-KSCs and KSCs.

Materials and Methods
PBLs
PBLs were obtained via leukapheresis from 2 patients preparing for bone marrow transplantation at Norris Cancer Hospital, USC School of Medicine, Los Angeles, CA, USA. Mononuclear cells (MNCs) were separated by centrifugation over Ficoll-hypaque (1.077 g/ml) (Organon Teknika Corporation, Durham, NC, USA). The banded cells were collected, washed twice with calcium (Ca^{2+})- and magnesium (Mg^{2+})-free Hank's balanced salt solution (HBSS) (Gibco, Grand Island, NY, USA), and cryopreserved with 10% dimethyl sulfoxide (Tera Pharmaceuticals Incorporated, Buena Park, CA, USA) in pentastarch (hydroxyethyl starch) serum albumin (Dupont, Wilmington, DE, USA) at concentrations of 20–50 million cells/ml and kept in liquid nitrogen. At the time of activation, frozen MNCs were thawed quickly in a 37°C water bath and suspended in complete medium (CM) comprising of RPMI1640 (Gibco) with 0.1 mM nonessential amino acids and sodium pyruvate (Microbiological Associate, Walkersville, MD, USA), 5×10^{-5} M 2-mercaptoethanol (Sigma, Saint Louis, MO, USA), 5% heat inactivated fetal calf serum (FCS) (Flow Laboratories, McLean, VA, USA), 1% L-glutamine (Irvine Scientific, Santa Ana, CA, USA), and 100 U/ml penicillin with 100 μg/ml streptomycin (Irvine Scientific). The cells were then washed twice, counted and diluted to 1 million cells/ml all using CM.

rIL-2 activation

In accordance with described protocols, 1 million MNCs/ml were treated with 1000 units of rIL-2 (kindly donated by Cetus, Emeryville, CA, USA) and immediately incubated under 5% CO_2/37°C for 4 days to generate LAK-cells [6]. These LAK-cells were then harvested, washed, counted, and their concentrations adjusted to 2.5 million cells/ml all using CM in preparation for chromium (^{51}Cr) release assays; LAK-cells were our effectors (E).

Target cells

Vascular ECs, MΦ and AIDS-KS10/-KS21 cells were used as ^{51}Cr labeled targets (H). KSCs were kindly donated by Zaki Salahuddin and Suji Nakamura, USC School of Medicine; the cells were developed at USC from KS lesions of AIDS patients at Los Angeles County General Hospital, CA, USA. All KSCs were cultured in 75 or 150 ml gelatinized, sterile culture flasks (Corning Glassware, Corning, NY, USA) containing 15–30 mls of KS medium (KSM). KSM comprised of RPMI1640 supplemented with 15% FCS, 4% 0.22 μm filtered nutridoma (Boehringer Mannheim, Indianapolis, IN, USA), and 20% activated CD4+ T-cell conditioned medium (T-cell CM) [21] (also kindly provided by Zaki Salahuddin and Suji Nakamura). Every 2–3 days, KSCs were renewed with 15–30 mls of fresh KSM. ECs harvested from human umbilical vein were similarly cultured in EC medium (ECM) comprising of CM supplemented with 4 mls of nutridoma and 30–45 μg/ml of endothelial cell growth supplement (Collaborative Research Incorporated, Bedford, MA, USA). As with KSCs, every 2–3 days, ECs received 6 mls of fresh ECM. When KSCs/ECs were confluent, they were trypsinized using 2 × trypsin-EDTA (Irvine Scientific) in Ca^{2+} and Mg^{2+}-free phosphate buffered saline (PBS), washed in CM, subcultured or cryopreserved. MΦ were isolated by culturing 2 million MNCs with 20 mls CM in 75 ml culture flasks for 2 hours under 5% CO_2/37°C. Following incubation, MΦ were separated by discarding nonadherant cells and harvesting adherent MΦ by means of a cell scraper (Baxter, McGaw Park, IL, USA), washed and counted all in CM.

mAb

mAb RH1-38 against LFA-1 was kindly supplied by Robert Hall [13], Guthrie Research Institute, Sayre, PA, USA. The Ab was added in dilutions of 1:100 and 1:400 per 100 μl using HBSS. Normal mouse serum (NMS-1) was employed as a control at identical dilutions.

^{51}Cr labeling

Concentrations of ECs, MΦ, AIDS-KS10/-KS21 were adjusted to 1–2 million cells/ml in CM and labeled using 500 μCi of $Na^{51}CrO_4$ (NEN Research Products, Boston, MA,USA) for 2 hours under 5% CO_2/37°C. After incubation, they were washed thrice, counted, and their numbers adjusted to 1 million cells/ml all using CM.

^{51}Cr release inhibition assay

The procedure for ^{51}Cr release assay was modified due to mAb additions as previously shown [10,11]. Briefly, to 96-well flat-bottom plates (Corning Glassware) were dispensed 0.25 million LAK-cells/well/100 μl. Identical number of these wells then received 100 μl of one of the following reagents: RH1-38 at 1:100 (E:Ab), RH-138 at 1:400 (E:Ab), NMS-1 at 1:100 (E:Ab), and NMS-1 at 1:400 (E:Ab). Plates were then left at 4°C for 30 minutes to permit Ab to E binding. Subsequently, single types of H were pipetted at 50:1 E:H ratio into equal number of E with Ab containing wells at every four predesigned E:Ab ratio. Maximum E strength was measured by incubation of E and H alone at 50:1 ratio; maximum isotope release was measured by incubation of H with 0.1 N HCl alone; and spontaneous release was measured by incubation of H with CM alone. Plates were then exposed to 5% CO_2/37°C for 4 hours. The culture supernatants were harvested using the Skatron Titertek System (Skatron A.S. Libergyn, Norway) and counted in a gamma counter (Packard Auto-Gamma-500; United Technologies Packard, Laguna Hills, CA, USA). The percentage (%) specific lysis was calculated by the formula:

$$100 \text{ X } \frac{\text{experimental count}(\text{cpm}) - \text{spontaneous count}(\text{cpm})}{\text{maximum count}(\text{cpm}) - \text{spontaneous count}(\text{cpm})}$$

All determinations were made in at least triplicate and data calculated as mean ± standard error of mean (SEM). Experiments were repeated at least once.

Authors's Contributions

Author 1 initials performed all laboratory procedures, conducted all experimental research, compiled a thorough literature survey, and drafted the manuscript for submission. Author 2 initials provided the funding, facilities, sources of reagents, experimental direction and manuscript peer review.

Acknowledgments

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