Histological and Immunological Features of Primary Kaposi's Sarcoma: Evaluation before and after Chemotherapy

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Forty-one patients with primary Kaposi's sarcoma (KS) have been evaluated clinically, histologically and immunologically at the time of diagnosis. There was no correlation between histological and immunological features. Moreover, the disease did not appear to be related to particular HLA phenotypes. The T4/T8 ratio was augmented. Leu 7+ cells were also significantly increased. The last 15 patients who received chemotherapy were recently reevaluated after treatment and an increase in B lymphocytes was observed. We also observed that spindle-shaped cells (SSC), which appear later in the histopathological course of the disease, disappear first during chemotherapy, concomitantly with the increase in B cells. We conclude that the course of the disease appears to be controlled by the host's immune response, though there is no clear correlation between histological and immunological evolution. Several immunological features differentiating it from AIDS associated KS have been found. *Key words: Histopathology; Lymphocyte subsets; HLA.*
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The main cutaneous histological characteristics of the multifocal progressive angioproliferative KS are the same in all the four clinical and epidemiological types (Classical Mediterranean, Middle-African, Primary Immunodeficiency-Associated, Epidemic AIDS-Associated) (1). They are proliferating processes of both differentiated (blood and lymph vessels) and undifferentiated (SSC bundles) structures. The different combinations of these features allow to recognize different stages (2–5).

The presenting stage, patch stage, shows empty vascular formations irregular in shape and size throughout the dermis, lined by thin and spaced endothelial cells; patchy lymphoplasmacellular infiltrates are seen in close relationship with the vascular structures (lymphatic channels?). Some SSC and extravasated erythrocytes (EE) are present near the vascular spaces. In the plaque stage there are much more SSC arranged in small clusters among which some slits with erythrocytes are present. The jagged vascular spaces and the lymphoplasmacellular infiltrates are still evident. Some EE, siderophages and a proliferation of apparently normal small haematic and lymphatic vessels can also be seen. In the late stage, the major finding is the presence of interweaving fascicles of SSC, located in reticular and deep dermis in nodular pattern with angioproliferative lesions at the periphery of the nodules. A great amount of slits filled with erythrocytes can usually be detected among SSC. There are numerous EE and siderophages among SSC and in the stroma around the nodules. From an immunological point of view, AIDS-associated KS is characterized by an inverted T4/T8 ratio, while in primary KS this is often increased (6, 7).

The aim of this study was to confirm in a larger group of patients the immunological alterations previously reported (6) and to assess whether they are related to the histological stage. Moreover, some of the patients were reevaluated after chemotherapy, looking for modifications of the histological and immunological pictures.

PATIENTS, MATERIALS AND METHODS

Histology

Forty-one patients (26 males and 15 females; mean age 69.2± 10.5 years, range 47–90) with the classical Mediterranean type of KS lasting from 5 to 120 months (mean 41) were classified histologically on the basis of the following criteria: 1) proliferation of SSC, 2) vascular formations, 3) inflammatory reaction, 4) extravasated erythrocytes, and 5) haemosiderin deposits. Each point received a score from 0 to 3. Fifteen of these patients were histologically reevaluated with the same criteria after intralesional and/or systemic chemotherapy (3 patients received only intralesional treatment, 9 only systemic and 3 both).

From 6 patients (3 with nodular KS treated with intralesional Vincristine (8) and 3 with the local aggressive form treated with both intralesional and systemic chemotherapy) second biopsies were taken from the injected area during complete clinical remission; only nodular exophytic lesions were treated. A total of 30 specimens was obtained. Twelve patients with the local aggressive form (plaques and nodules) were given monochemotherapy with Vinca Alkaloids (9) or polychemotherapy according to standard protocols (ABV or EBV) (10, 11); three of them received also intralesional chemotherapy as reported above. From all of them, second biopsies were taken when complete clinical remission had been achieved, while from 4 a biopsy was also taken during partial clinical remission (more than 50% reduction of initial lesions). In systemically treated patients both the first and the second histological evaluation was performed from the most involved area.

Since the lesions evolve independently from each other, we chose plaque lesions because i) they can be partially excised, leaving the remainder for a later histological evaluation, and ii) they show proliferation of SSC in appreciable amounts, suggesting an almost or completely mature histogenetic process. Thus, post-chemotherapy biopsies were taken from the same residual plaque lesions.

Immunology

Lymphocyte subpopulations. Peripheral blood lymphocytes were obtained from heparinized blood by centrifugation on a gradient of Ficoll-Hypaque (Biotest Laboratories) (12) from 41 patients. The cells were then washed three times in PBS and processed for the detection of membrane markers. A panel of monoclonal antibodies (OKT3, OKT4, OKT8, OKT6, OKIa1; Ortho Pharmaceutical Corp.) was used to recognize T cell subsets and Ia+ cells, while another monoclonal antibody (Leu 7; Becton Dickinson) was employed for NK cells. All these tests were performed with an indirect immunofluorescence method (13), using a fluorescein conjugated goat anti-mouse antibody (Meloy Laboratories) as second reagent. B-lymphocytes were recognized by a direct immunofluorescence assay (14), using a fluorescein-labeled F(ab)2 to human immunoglobulins (Kallestad Laboratories). Cell counts were taken with an UV light-equipped microscope (Dia-Lux; Leitz) at 1000 magnifications. Thirty healthy subjects formed the control group. In the 15 patients, whose histology was reanalysed after chemotherapy, a post-chemotherapy immunological evaluation was also carried out.

HLA phenotyping. Unfractionated lymphocytes obtained as above were HLA-phenotyped for A, B and C antigens by the microdroplet cytotoxicity assay (15). DR antigens on B lymphocytes separated by spinning the unfractionated lymphocytes on a Ficoll-Hypaque gradient after rosetting with AET-treated red blood cells (16) were studied with the same method. The antisera recognized 42 class I and 8 class II specificities and were purchased both from commercial suppliers (UCLA tissue typing laboratories; Biotest Laboratories) and from different Italian tissue typing laboratories. The control population was made of 117 subjects with a regional provenance similar to that of the patients.

Statistical analysis

The Student's *t*-test for unpaired observations and the X square test were used when appropriate according to standard statistical methods (17).

RESULTS

Intralesional chemotherapy always induced complete clinical remission and histological changes, including the disappearance of SSC, bizarre-shaped and lymphatic vessels, EE

Table I. *Histological evaluation of KS before, during and after treatment*

Scores based on the relative percentages of histological elements forming the single lesion. SSC = spindle-shaped cells; VF = vascular formations; IR = inflammatory reaction; EE = extravasated erythrocytes; HS = haemosiderin deposits

	Before treatment					During treatment					After treatment				
Pts	SSC	VF	IR	EE	HS	SSC	VF	IR	EE	HS	SSC	VF	IR	EE	HS
1	1	2	3	2	1	–	–	–	–	–	0	1	0	0	1
2	1	2	1	0	1	–	–	–	–	–	0	1	1	0	0
3	3	2	1	3	2	–	–	–	–	–	0	1	0	0	1
4	1	2	2	0	0	–	–	–	–	–	0	1	0	0	0
5	1	3	2	0	1	–	–	–	–	–	0	1	0	0	2
6	3	1	1	3	1	–	–	–	–	–	0	0	0	0	2
7	3	2	1	2	0	–	–	–	–	–	0	0	0	0	0
8	3	1	1	3	2	–	–	–	–	–	0	1	0	0	2
9	1	3	1	0	2	0	2	1	0	2	0	1	0	0	2
10	2	2	2	1	1	0	2	1	0	1	0	1	0	0	1
11	2	3	2	1	2	0	2	0	0	2	0	1	0	0	2
12	3	3	2	2	1	1	2	1	0	2	0	1	1	0	1

and inflammatory infiltrates. Some haematic capillaries and haemosiderin deposits free in the dermis and in the cytoplasm of macrophages were still present. Minor reticular dermal scarring processes residuated. Table I shows the biopsies from 12 patients who received systemic chemotherapy (3 of them also received intralesional chemotherapy). All of them were in complete clinical remission when histologically reevaluated; the patients 9 to 12 were also biopsied in the partial regression stage: in these, we saw (2nd column) that bizarre-shaped and lymphatic vessels, SSC and EE were the first in time to disappear, while there was a slower regression of normal-shaped haematic neoformations and mononuclear infiltrates. The third column shows the histological findings of the patients in complete clinical remission. In 10 out of 12 patients, vascular formations formed by haematic capillaries throughout the entire dermis were still present in small amount; in 2 we observed a certain degree of inflammatory reaction, composed of small patches of lymphocytes and histiocytes, whereas in all the patients SSC and EE were absent. Haemosiderin deposits and dermal fibrosis residuated after treatment.

Immunological features before treatment were characterized by a slight decrease in total T cells, mainly due to a reduction in T8+ cells, with an increased T4 to T8 ratio. Leu7+ cells were greatly augmented, but there were no major modifications in the percentages of T6+, Ia+ and B lymphocytes (Table II). Since the mean lymphocyte counts were comparable in the patients and in the control group, these findings also reflect changes in absolute numbers.

The immunological features of the 15 patients who underwent chemotherapy were not significantly different from those of the other patients, the assignment to treatment being decided only on a clinical and histological basis. When they were re-evaluated after treatment, a significant increase in B lymphocytes was noticed. T cells were also augmented, because of an increase in T4+ cells, but this difference was not statistically significant, because of the large standard deviation (Table III); thus the T4/T8 ratio did not appear to change after chemotherapy.

HLA antigen frequencies for the patients are shown in Table IV. There were no significant differences between patients and controls for A, B, C and DR antigens.

Table II. *Percentage of lymphocyte subsets and helper/suppressor in the patients studied*

	Patients	Controls	*p*
T3	63.2±15.1	71.7±9.7	<0.05
T4	43.5±15.9	44.1±9.0	NS
T8	20.4±8.2	27.5±3.9	<0.001
T6	1.3±4.7	1.3±1.7	NS
Ia	4.5±4.4	4.6±3.5	NS
B	9.2±7.0	9.3±4.9	NS
Leu7	25.2±10.6	13.6±6.3	<0.001
T4/T8	2.6±1.5	1.6±0.4	<0.01

DISCUSSION

Since KS is a multifocal disease, in which cutaneous lesions begin and progress independently in time (2–5), the histological evaluation is useful or even mandatory for the diagnosis, but it is of limited value in the clinical classification and staging. The same considerations apply to the histological assessment of chemotherapy efficacy. Therefore we made biopsies from the same lesions, as explained above. We found that, during chemotherapy, SSC, EE and bizarre-shaped lymphatic vessels disappear first, while neoformed haematic capillaries and inflammatory infiltrates show a slower pattern of regression. This observation suggests that less differentiated structures are more sensitive to chemotherapy. Clusters of capillaries scattered in the dermis persist in the residual lesions. Therefore it appears that histological regression is not concomitant with clinical remission. The vascular formations could perhaps be considered as anatomical remnants of a preexisting angioproliferative process and not as an index of activity of the neoplasm. A further demonstration of this hypothesis is given by the fact that a clinical reappearance of the disease was never observed in our patients followed for a mean period of 22 months (range 3–48) after chemotherapy had been suspended. In consideration of our results, we think that, in complete clinical remission, SSC, EE, bizarre-shaped vessels and lymphoplasmacellular infiltrates are the main elements to be considered, when deciding if chemotherapy should be discontinued.

As far as immunological features are concerned, the major finding in our patients is an increased T4/T8 ratio. This result confirms our previous observations in a smaller group of patients (6) and could be interpreted as a modification induced by the tumor. This type of response is not seen in KS associated with AIDS (18) and once again it indicates that there

Table III. *Changes in lymphocyte subpopulations with chemotherapy. Data obtained in 15 patients*

	Before treatment	After treatment	*p*
T3	59.9±20.1	69.5±9.7	NS
T4	41.3±18.6	47.7±15.1	NS
T8	20.9±9.6	20.6±8.2	NS
Ia	4.2±3.0	6.5±4.6	NS
B	7.5±4.7	12.5±5.5	<0.001
T4/T8	2.6±1.7	3.1±1.7	NS

Table IV. *HLA antigen frequency (% of subjects expressing each given antigen)*

A	%	B	%	B	%	C	%	DR	%
1	27.8	5	–	47	–	W1	11.1	1	32.1
2	47.2	7	2.8	W48	2.8	W2	2.8	2	28.6
3	13.9	8	8.3	49	2.8	W3	5.6	3	10.7
9	5.6	12	2.8	W50	2.8	W4	27.8	4	7.1
10	–	13	2.8	51	19.4	W5	5.6	5	60.7
11	2.8	14	2.8	W52	–	W6	13.9	W6	10.7
23	2.8	15	11.1	W53	–	W7	–	7	25
24	19.4	16	–	W55	–	W8	–	W8	10.7
25	2.8	17	2.8	W56	–				
26	25	18	19.4	W57	2.8				
28	5.6	21	2.8	W58	–				
29	2.8	W22	5.6	W59	2.8				
30	2.8	27	–	W60	–				
31	2.8	35	16.7	W61	–				
32	2.8	37	2.8	W62	5.6				
W33	–	38	11.1	W63	–				
W34	–	39	13.9	W64	–				
W36	–	40	–	W65	–				
W43	–	W41	–	W67	–				
W66	–	W42	–	W70	–				
W68	–	44	13.9	W71	–				
W69	–	45	–	W72	–				
		W46	–	W73	–				

are probably two different aethiopathogenetic mechanisms for KS. Interestingly, B lymphocytes were not increased in untreated patients, but rose significantly in those who were recontrolled after chemotherapy and in whom the reduction of the neoplastic mass had been achieved. This increase in B cells may be due to the increased T4/T8 ratio. The fact that it is not seen before treatment, though there is an already increased T4/T8 ratio, could be due to the production of blocking factors able to prevent B cell proliferation by the tumor. The raise in B lymphocytes after chemotherapy could be relevant, if one thinks that antibody-mediated immunity plays a role in controlling neoplastic growth. If this is true an increased number of B cells could be in part responsible in maintaining the remission stage achieved by treatment.

Leu 7+ cells were also significantly increased in our patients, indicating a recruitment of NK cells, which is a well-known immune response to tumors (19). It is interesting to note that this type of response has also been found in subjects with the lymphoadenopathy syndrome (LAS) (20). Why NK mediated immune responses are among the first mechanisms of immunological activation seen in these diseases remains unclear. However, the fact that this change can be detected by a simple and rapid method, such as the one described, could make it a valuable tool in the routine evaluation of these patients.

Finally, we wish to mention that no association with HLA antigens was found. These data are at variance with those of other authors who reported both an association with class I (21) and class II antigens (7, 22). The latter association mainly interests the DR5 antigen, which in our group appears to have only a marginally increased frequency. We think that this difference can be explained on one hand by the lower frequency of DR5 in healthy members of the populations of those studies, and on the other by the fact that AIDS-associated KS could be immunogenetically different from primary KS. However, the fact that the Italian population has a high DR5 frequency and that Italian ascent is

statistically more frequent in patients with KS living in America (7) deserves further investigation. For instance, an increased frequency of a supratype, as suggested for other diseases (23), should be looked for in KS, and further work is in progress in our laboratory to elucidate the possible immunogenetic factors involved in KS, also because a familial occurrence of this disease has sometimes been observed (24).

On the basis of our data, we conclude that while there are no correlations between immunological and histological features some of these appear to show very clear modifications with chemotherapy. Finally, it is possible to say that primary KS is seen in patients with marked immune responses which involve many components of cell-mediated immunity. Such immune responses might be able to partially control the neoplastic proliferation and could therefore be responsible for the relative benign course of the disease.

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