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Quantitative and Functional Abnormalities of Total T Lymphocytes in Relatives of Patients with Hodgkin's Disease*

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Abstract—*Seven patients, long-term survivors of Hodgkin's disease, and 24 of their relatives (parents, siblings and children), together with normal controls were studied for percentages, absolute counts and mitogen-proliferative responses by means of monoclonal antibodies, E rosette technique and* in vitro *cultures with PHA, ConA and PWM. The aim of the study was to ascertain whether the impaired cell-mediated immunity of Hodgkin's patients was also present in relatives in order to elucidate the still debated etiology of the defect and of the disease (congenital? environmental? infectious?). The results show that both Hodgkin's patients and their relatives have a significant decrease of total T cells (as T3+, T11+ and E rosette-forming cells) in peripheral blood and a significant impairment of polyclonal responses to all the mitogens employed. The Leu-7+ cells (i.e. a consistent amount of natural killer cells) are significantly increased only in the Hodgkin's patients but not in their relatives. The T cell subpopulations (T4 and T8), B cells and monocytes do not show any difference between the patients, their relatives and normal controls. Our results seem to support, at least in part, the presence of a common defect of T cell lineage both in patients and in their relatives, but its etiology still remains uncertain (genetic? environmental?).*

INTRODUCTION

HODGKIN'S disease (HD) is different from the other malignant lymphomas (ML) in its immunological aspects because of its peculiar defects of T cell-mediated immunity [1]. Also the high frequency of familiar cases is unusual in ML, whereas the risk of HD is high for relatives and much more so for siblings of HD patients than for the control population [2-7]. Contradictory data have been obtained so far concerning the relationship between HLA antigens and the risk of HD [6-9]. Recently Svejgaard *et al.* [10] found an increase in the frequency of HLA-A1, B5, B8 and B18 in HD patients and others [11] have shown a correlation of HLA-Aw19 with a poor prognosis in patients with unfavourable age, stage or histology. Hors *et al.* [6], in 13 family cases of HD (seven pairs of affected sibs, four parents/children and two first cousins), found an excess of HLA identical patients (6/7) among sibs, which when compared with international data reaches a significant level, indicating that HLA complex could have an important role as a factor of susceptibility to HD.

The impairment of T cell-mediated immunity has been well documented [12] and consists of a reduced reactivity to recall antigens by skin tests [13], decreased E rosette formation [14-16] and mitogen-induced lymphocyte proliferation [17-19]. These parameters are affected both in untreated and in long-term survivors [20-21].

Recently T cells in peripheral blood of patients with HD have been studied by means of monoclonal antibodies specific for lymphocyte antigens [22] with contrasting results. Our group found a moderate imbalance of T4/T8 ratio

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together with a decrease of E rosette-forming cells (ERFC) in untreated patients with HD [23]. Other groups [24], however, were not able to find significant differences as far as the T cell subpopulations were concerned.

The quantitative and functional alterations of T cells in peripheral blood and consequently the defect of T cell-mediated immunity have been attributed to different etiologic factors; absolute lymphocytopenia, selective depletion of T cells, intrinsic T cell defects, sequestration of immunocompetent cells in involved tissues, activation of non-specific suppressor cells and immunosuppressive plasma factors have all been indicated as being responsible. One fact seems to emerge: the immunodeficiency associated with HD can be considered as multifactorial in its etiology.

With regard to the plasmatic factors, it is worth noting that sera of HD patients in certain circumstances can inhibit proliferative responses to polyclonal mitogens and contain E rosette-inhibiting factors and lymphocytotoxins [15, 25-27]. The latter can be found also in relatives of patients with HD in a significantly higher percentage than in normal controls (35.2 vs 3%) [28]. Also in experimental models of HD (SJL/J lymphoma in mice) plasma immunosuppressive factors have been found [29].

From these data HD appears to be a disease where a mis-regulation of the cell-mediated immune response has an unknown etiology and seems possibly to be related to intrinsic defects of both its lymphoid lineage and its distribution but also to extrinsic and environmental factors.

To ascertain whether congenital or familial factors either independently from or together with environmental agents could be important in generating both the immune defect and HD we examined peripheral lymphocytes from long-term survivors of HD, without therapy for at least 4 yr, and peripheral cells of their relatives, to study the percentage and the absolute counts of T cells and their subpopulations and their responses to polyclonal mitogens.

MATERIALS AND METHODS

Patients

Seven long-term survivors of HD (four males, three females), aged 28-40 (mean age 32 yr), were studied. Their characteristics with regard to sex, age, time of diagnosis, clinical stage [30], histology [31] and treatment are listed in Table 1.

Relatives

The relatives came from the seven respective families (indicated in the figures as F1-F7), totalling 24 persons (F1 being composed of one only — the sister of the patient). There were nine males and 15 females, aged 6-74 yr (mean 32 yr); 20 of them lived in the same environment as the patients, and the other four did not (all females, two aged 31 and two aged 35 yr).

Among the relatives seven were parents, four children and 13 siblings. None of them showed signs of any disease at the time of the study and they gave their consent to the study together with the patients.

Controls

Eight normal controls, age- and sex-matched to the HD patients, were studied for the lymphocyte populations. Ten with the same characteristics were used for the study concerning the responses to polyclonal mitogens.

Methods

The heparinized peripheral blood was processed by the usual methods to obtain mononuclear cells [density gradient on Lymphoprep (Nyegaard, Oslo), then depletion of adherent cells on plastic capsules]. The purified lymphocytes were washed

Table 1. Patients long-term survivors with Hodgkin's disease. Clinical data

No.	Sex*	Age (1984)	Year of diagnosis	Stage†	Histology‡	Treatment§	Years without therapy
1	M	40	1972	II A	LP	C	10
2	M	34	1972	III B,S	MC	C,R	5
3	M	30	1974	III B,S	MC	C	8
4	F	35	1974	III A	MC/NS	C	9
5	F	30	1975	II A	NS	R	6
6	F	29	1976	II A	NS	R	7
7	M	28	1977	III A,S	NS	R	6

*Sex: M = male; F = female.
†Ann Arbor, 1971.
‡Rye. LP = Lymphocyte predominance; MC = Mixed cellularity; NS = Nodular sclerosis.
§C = Chemotherapy, MOPP; R = Radiotherapy, mantle and inverted Y, plus spleen.

twice in RPMI 1640 supplemented with 20% fetal calf serum and resuspended at a concentration of 10×10^6 cells/ml; 50 μl were incubated with a series of monoclonal antibodies (equal volume of the appropriate dilutions) at 4°C for 30 min. The monoclonal antibodies were: series OK (Ortho Raritan N.J., USA): T3 (total T cells), T11 (receptor for E rosette, total T), T4 (prevalently helper/inducer), T8 (prevalently suppressor/cytotoxic), M1 (monocytes, macrophages) and Ia1 (B cells); series Leu (Becton-Dickinson, Mountain View, CA, USA): Leu7 (mostly natural killer cells). After incubation the cells were washed three times with RPMI, incubated at 4°C for 30 min with an anti-mouse IgG conjugated with fluorescein isothiocyanate (Cappel), washed three times with RPMI and viewed using a Leitz Ortholux microscope equipped with Ploem UV epiillumination [23].

The E rosette technique was performed according to the usual methods described elsewhere, using purified lymphocytes in RPMI supplemented with 20% fetal calf serum and considering the E rosette as a lymphocyte surrounded by at least three sheep erythrocytes [32].

The cell cultures for the determination of the response to polyclonal mitogens were grown (in triplicate) in microtiter plates containing 1.5×10^5 peripheral lymphocytes (non-purified and thus containing also adherent cells) in HEPES-buffered RPMI 1640 medium. The cells were put in contact with phytohemagglutinin (PHA-M, Difco) (5, 10 and 20 γ in 200 μl medium) for 72 hrs, concanavalin-A (Con-A, Calbiochem) (1 and 2 γ in 200 μl medium) for 72 hr and pokeweed mitogen (PWM, GIBCO) (1:100 and 1:200 of the initial dilution in medium) for 120 hr. They were pulsed with 1 μCi ([^{3}H]thymidine) for the final 14–18 hr of the cultures, then harvested on a multiple automated sample harvester (Skatron) and counted in a liquid scintillation counter (Packard). The details have been described elsewhere [33].

The statistical analysis were performed using Student's t test for the difference between the means with significant levels of 0.05, 0.01 and 0.001.

RESULTS

The results for the percentages and the absolute counts of the T cells studied by monoclonal antibodies and the E rosette technique are shown in Fig. 1. It is worth noting that total T cells (ERFC, T3+ and T11+ cells) are significantly reduced in HD patients and their relatives considered as a whole population when compared with normal controls with regard to percentages (for HD patients 52.5 ± 12.4, 52.0 ± 13.4 and 52.7 ± 12.6, for relatives 57.2 ± 12.2, 60.4 ± 9.1 and 55.3 ± 14.2 respectively, vs 70.0 ± 6.0, 72.5 ± 9.0 and 77.5 ± 8.0 for normal controls).

As far as the absolute counts are concerned OKT3+ and OKT11+ cells are significantly lower in HD patients and their relatives than in normal controls (HD 1699.2 ± 464.3 and 1653.1 ± 298.6, relatives 1501.0 ± 715.3 and 1306.0 ± 490.5 vs 2078.0 ± 429.0 and 2465.0 ± 488 for normal controls), whereas ERFC are lower than in controls but not significantly (1658.0 ± 364.0 for HD and 1404.9 ± 537.0 for their relatives vs 1719.0 ± 355.0 for normal controls).

OKT4+ and OKT8+ cells do not show any significant difference in either HD patients or their relatives compared with normal controls, so that the T4/T8 ratio is in the normal range. Also OKM1+ and OKIa1+ cells are in the normal range for HD patients and their relatives.

Leu7+ cells (where a majority of natural killer cells seems to exist) in HD patients are significantly increased where both percentage and absolute count are concerned (23.5 ± 12.0 vs 14.3 and 782.0 ± 44.0 vs 436 ± 81.0) when compared with data of their relatives and of normal controls. Relatives do not exhibit significant differences with regard to the Leu7+ subset of cells when compared with controls.

The comparison of the results obtained in the individual patients with the means of their families are shown in Fig. 2. The decrease of T3+, T11+ and ERFC cells is more evident when the percentages are taken into consideration, but the absolute counts do show that both patients and their relatives are below the normal range with the exception of F1, composed of only one member, the other families having a minimum of three (excluding the patient) (three families) to a maximum of six members (F3). In each family group individual subjects had a decreased level of T3, T11 and ERFC cells, varying from a minimum of one out of three members in F4 (but two out of three had a lower than normal ERFC) to a maximum of four out of four (F2, all from the same environment) and of five out of six (F3, all from the same environment).

It is worth noting that in F5 two of the members are children (6 yr, male and 9 yr, female); the first one was born 15 months after the completion of radiotherapy and the second one was born at the beginning of the disease when the mother had been given only two doses of 250 rad in the mantle region (the treatment was begun during pregnancy due to a serious mediastinal syndrome): both showed a significant reduction of T3+ (46 and 48%), and T11+ cells (26 and 44%) and one

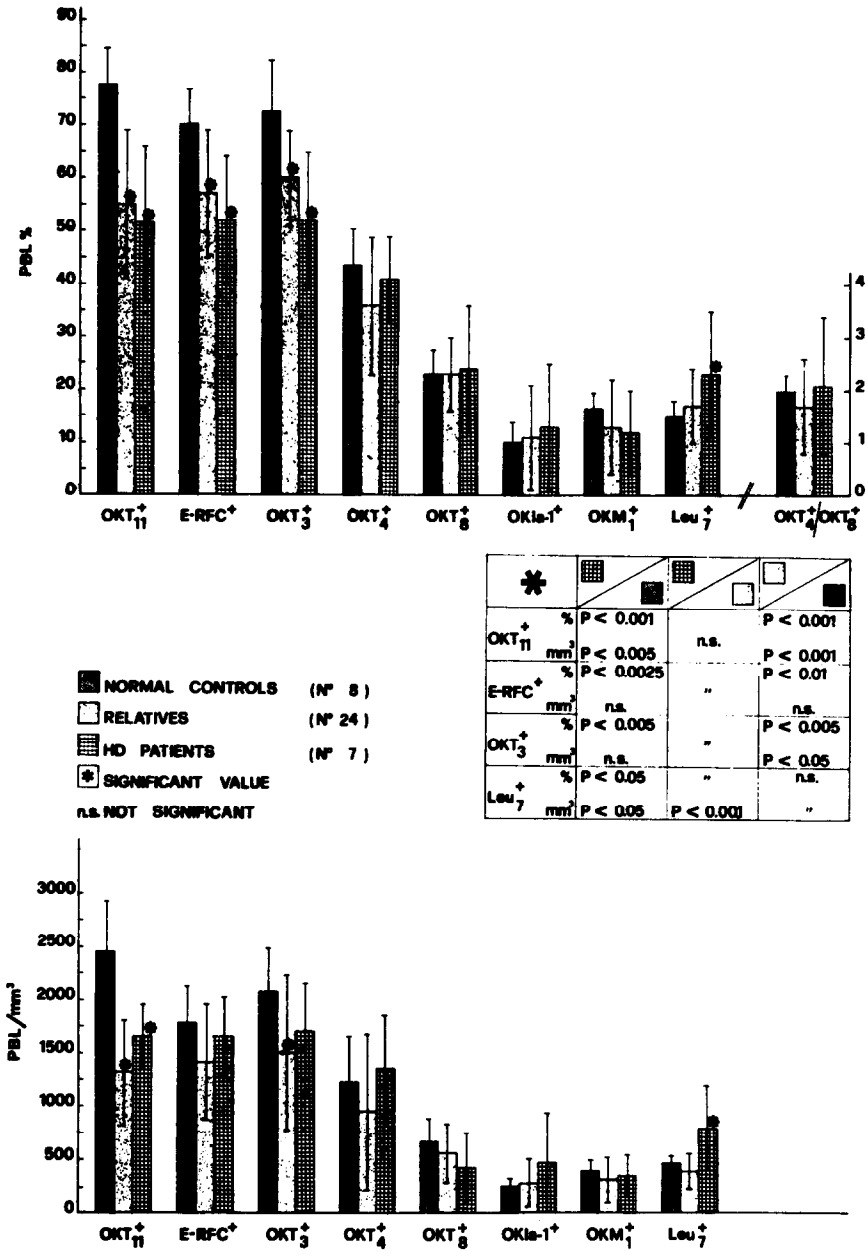

Fig. 1. Percentages and absolute counts of peripheral lymphocytes studied by monoclonal antibodies and the E rosette technique in Hodgkin's disease patients, their relatives and normal controls.

only showed a significant reduction of the ERFC cells (60%). On the contrary, the two children in F4 (9 yr, male, born 11 months after the completion of the MOPP cycle and 11 yr, female, born 10 months before the disease was diagnosed) exhibited less severe defects of T cells. The male has absolute counts of T3 and T11 cells lower than normal and the female has a lower than normal percentage of ERFC cells. All the parents studied (both parents in F2, F6 and F7 and the mother in F3) showed a significant decrease in T3, T11 and ERFC cells in both percentages and absolute counts, also taking into account that in older subjects total T cells can be decreased when compared to the normal adult population. In fact the fathers studied in this paper were respectively 74, 58 and 66 yr old and the mothers 60, 53, 58 and 53 yr old and cannot therefore be considered as very old people (except for the 74-yr-old father).

Among the relatives not living in the same environment (sisters of F1, F4, F5 and F6), F1 shows a decrease in ERFC (percentage and absolute count), F4 of T3+, T11+ and ERFC (percentages and absolute counts), F5 of T3+ and T11+ (percentages and absolute counts) and F6 of T3+ and T11+ (absolute counts) and ERFC cells (percentages and absolute count).

Seven out of 24 relatives show increased values of Leu7 when compared to normal controls (two in the F2, father and sister; two in F3, brother and

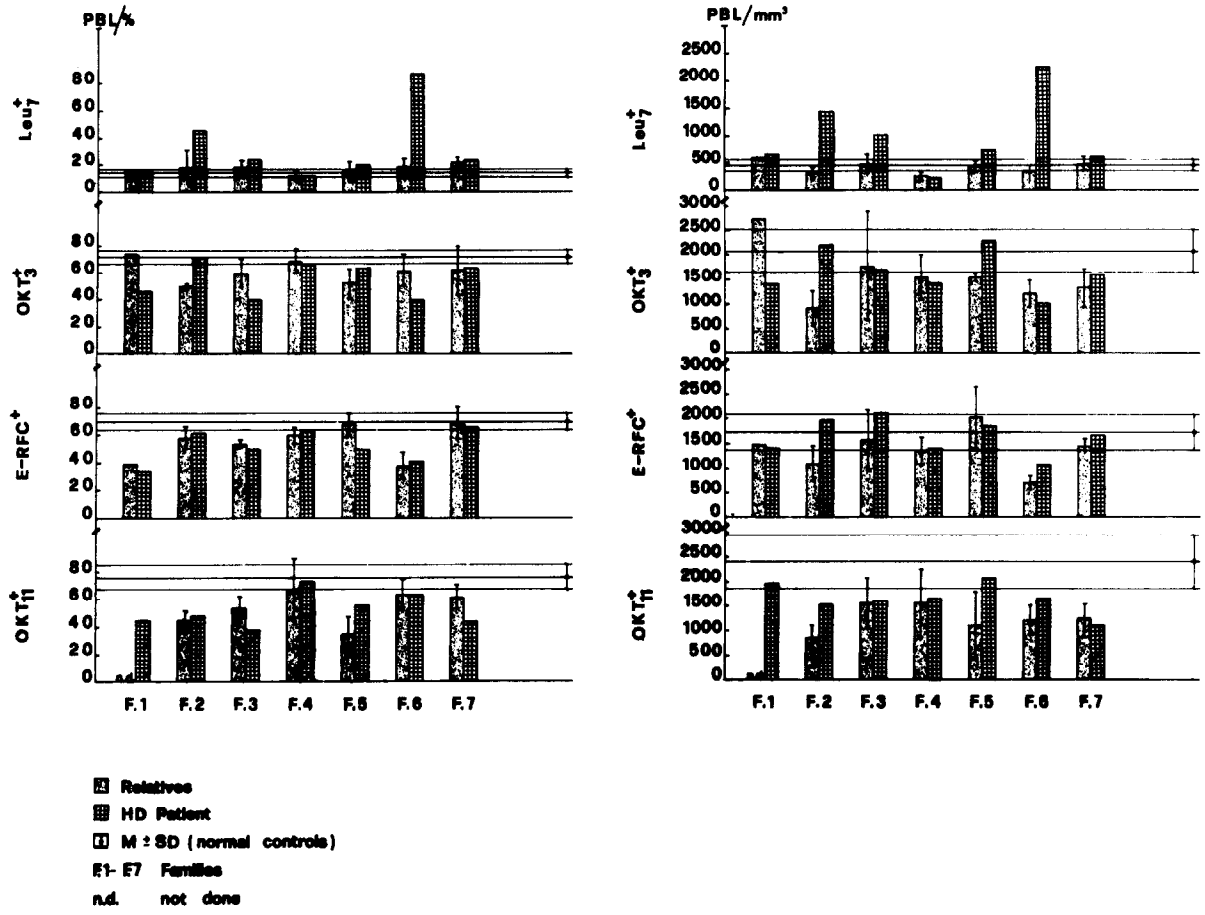

Fig. 2. A comparison of the percentages and absolute counts of total T cells (ERFC, T3+ and T11+) and of Leu 7+ cells of peripheral blood, between individual patients with Hodgkin's diseases and their families.

sister; one in F5, sister, not living in the same environment; and two in F7, father and brother).

The polyclonal mitogen responses are shown in Fig. 3.

The cpm $\times$ 10^3 concerning the different responses to the various concentrations of PHA, ConA and PWM indicate that the cells of HD patients and of their relatives, considered as a whole population, behaved in the same way, having a complete hyporesponsiveness to all mitogens considered, significantly lower than in normal controls; only the responses to sub-optimal concentrations of PHA (5 γ) were lower than in the normal controls but not significantly. So the responses of the familial group were generally higher than those of the patient but always significantly lower than those of the controls. Only one patient responded at the lower level of the normal range for PHA (but not for ConA and PWM); some individual relatives (eight) responded normally to mitogens but in each family group there were subjects with this kind of defect (from a minimum of one to a maximum of four), and the trend of each family was the same as the patient: when the patient had very low responses, more relatives had low responses to polyclonal mitogens.

DISCUSSION

Our data concerning the immunological responses of HD patients confirm the results presented in the literature (see Introduction). Also the studies with specific monoclonal antibodies for T cells in the peripheral blood confirm that total T cells are low and that the percentage and absolute counts of helper/inducer and suppressor/cytotoxic are normal in most of the patients [24]. It is noteworthy that in our study the patients were long-term survivors and that four of them were given radiotherapy, of which the effects on the number and functions of lymphocytes [34] can last longer than 10 yr.

The most interesting fact is that the relatives of the HD patients also had had similar totals of T cells and responses to polyclonal mitogens to the patients themselves. The values are statistically significant and do not show any difference between HD patients and their relatives, whereas both HD patients and their relatives were significantly different from normal controls in

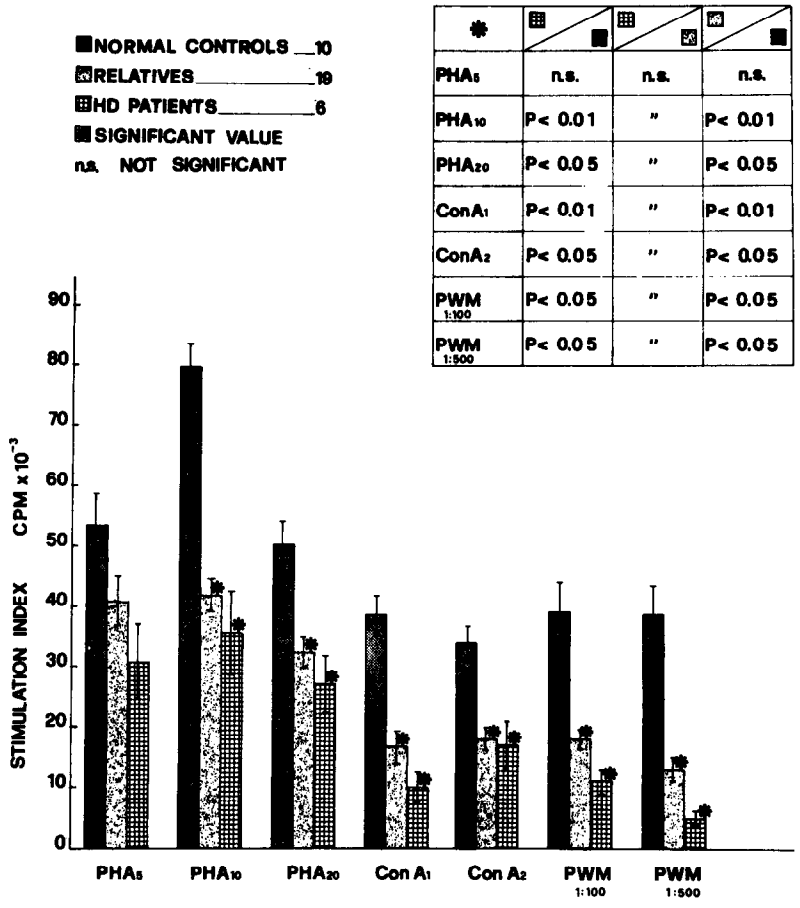

Fig. 3. The responses to polyclonal mitogens (PHA, ConA and PWM) at different concentrations of the peripheral lymphocytes from patients with Hodgkin's disease, their relatives and normal controls.

the same parameters. Moreover, considering individual data of the relatives, a large number of them had defective values of percentages and absolute counts of total T cells and responses to polyclonal mitogens, with no differences between individual subjects living in the same environment and those living elsewhere (even though there were only four of the latter). Subjects showing immune defects were present among parents, siblings and children (in two of the latter the effects of therapies carried out during pregnancy of the affected mothers could be seen). The only difference between HD patients and their relatives was in the Leu7+ cells (i.e. the subset of cells with a majority of natural killer cells): they were significantly increased in HD patients but not in the mean of their relatives (7/24 had increased values). The relatives behaved, as a mean, like normal controls when this parameter was considered. These data deserve some consideration.

Among the causes of T cell deficiency in HD, the sequestration of functional T cells from the blood to tissues has been considered by some authors [35]. This observation is based on the phenomenon of lymphocyte trapping in peripheral tissues, mainly in spleen and lymph nodes, after contact with antigens. Some antigenic contacts with viruses or neoplastic factors could induce HD patients to trap their T cells in peripheral organs with blood depletion (ecotaxopathy).

Some data could account for this hypothesis: presence of 'reactive' large T cells in the peripheral blood, normal response to PHA of spleen cells in HD, and mis-distribution of T-γ and T-μ cells [36] between lymph nodes and blood (T-γ low in tissues and high in blood, T-μ high in tissues and low in blood). Data about monoclonal antibodies for T cell subpopulations, however, do not seem to confirm this last result.

A congenital defect regarding the trend to a mis-distribution of T cells between blood and tissues could involve many members of the same family and could explain, at least in part, our findings. This could explain why, for example, the defect is present in parents, siblings and children, even if they are not living in the same environment or (for children) if they were born when the disease was in complete remission and after completion of the therapy. The difference between patients and healthy relatives could be found in the contact

with the 'noxious agent' (oncogene-dependent? virus? environmental factor?) acting on only one subject, bearing an enhancing or facilitating immunological background common to the entire family.

The increase of NK cells could be a sign of the reaction of the patients against the 'agent' of the disease. Moreover, familial cases of HD have often been described [6] and in these cases the same factor could have induced the disease in more than one member.

Nonetheless an infectious etiology has also been suggested for HD and clusters of patients have been found [37]. Thus the same etiological factor could act as an immunodepressive agent (virus?) on the same environment, inducing the same immune alterations in various subjects and causing the disease at random in one or only a few members of the same family (amount of infectious charge? better acceptance of the host background?).

An intrinsic defect of T cells has also been postulated and our data, mainly those concerning the responses to polyclonal mitogens, could account for it. The NK cells, which seem to derive from a lineage different from T cells, are not involved and in the affected subjects (HD patients) are high in response to the etiologic agent.

We did not search for lymphocytotoxin or other soluble inhibiting factors in the blood of the patients and their relatives; but it should be borne in mind that this parameter, indicating a humoral response against an antigenic etiological agent (or environmental and not primitively involved agent?), also has a typical familial behavior [28]. The same happens for lymphocytotoxins present in LES [38] or inflammatory bowel diseases [39].

The role of suppressor mechanisms in induction of T cell deficiency in HD is still under debate and hitherto a clear influence of cells with suppressive activity or of soluble factors producing cells (prostaglandins?) [40] in the induction of the immune defect in HD and in their relatives has not been clearly demonstrated. We did not perform functional studies on the suppressive activity of T cells in HD patients or their relatives; the only data we have concerning suppressor cells are those on T8+ cells in peripheral blood: these cells are commonly defined as suppressor/cytotoxic, but it is well known that they bear many different activities and that they cannot be considered as a 'pure' subset of cells without also performing functional tests; in any case, in this subset suppressor cells behaved normally in HD patients and their relatives. Interestingly, a recent hypothesis has been made concerning the significant impairment of T cell-mediated immunity in HD. The persistence of the various abnormalities also in long-term survivors cannot be attributed to chemotherapy or radiotherapy since they are not present in long-term survivors of non-Hodgkin's ML treated with comparable chemotherapy or radiotherapy; some authors [41] provide consistent data indicating that T cells of HD patients have an increased sensitivity to the regulation by suppressor cells and this way of responding to regulatory signals could explain why HD patients, untreated and long-term survivors, exhibit a decreased immune response. The defect could be familial and could also explain the behavior of the relatives of HD patients. On the other hand the increased sensitivity could be acquired and persist with HD but its presence in relatives could suggest an inherited genetical defect and studies on the sensitivity of relatives to T cell regulatory signals are planned to elucidate this aspect.

We did not perform HLA typing in our families but from previous data of other authors [6] the role played by HLA system seems to be important as a factor of susceptibility to the disease together with environmental factors: the HLA complex could induce an immune response defect, also demonstrated in healthy relatives, on which the agent of the disease failed to act. The susceptibility of siblings, with regard to the behavior of the HLA complex, appears to be bound to a recessive trait, as in experimental leukemias [42].

Of course, the HLA complex alone is not sufficient to induce the disease but certainly other genetical or environmental factors contribute towards it probably through a basis of an immunological deficiency.

In conclusion, from our data some considerations can be made: (a) quantitative and functional alterations of T cells in HD are confirmed also in long-term survivors, and this does not seem to be due to the treatments but probably to an intrinsic or acquired defect, characteristic of the disease; (b) the relatives of HD patients show the same quantitative and functional defects of total T cells, and this could indicate that familial factors together with environmental ones can influence the immune reactivity; (c) the NK cells are increased in HD patients even after many years following remission and the completion of treatment; this phenomenon can be explained by a reaction against neoplastic factors and its persistence could be a sign of effective control of the disease; (d) the relatives do not show any modification of NK cell amounts in peripheral blood, probably because they failed to contact the Hodgkin's agent; (e) no alteration of T cell subsets has been demonstrated in patients and in relatives, with the exception of a few individual cases showing a T4/T8 ratio at low levels of the

normal range; (f) the hypothesis that HD represents a disease with multifactorial etiology where inherited and/or familial factors (HLA complex, immunodeficiency of T cells, misdistribution of T cells between blood and tissues) together with environmental ones (viruses, neoplastic agents) can be combined or mixed variously to induce the real disease status appears to gain further support from our data, which contribute in a small way towards the knowledge of the immunosuppression of HD.

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