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Immune-deficiency in Hodgkin's Disease (HD): a Study of Patients and Healthy Relatives in Families with Multiple Cases

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Abstract—*In this study we evaluated some immunological features in both patients and healthy relatives within five families with multiple cases of Hodgkin's disease (HD). Such familial groups (at 'high risk' of HD), represent, in our opinion, a suitable opportunity to investigate the role of immune-deficiency in HD.*

The results obtained in the patient group confirm the well known persistent immune derangement in long term HD survivors.

Regarding the group of relatives, we found a pattern similar to that of the patients. In particular, a decrease in the T helper lymphocyte subset and a lower response to Con-A mitogen were detected, which were statistically significant.

These findings, confirmed in other studies of multiple case families, could support the hypothesis of a preexisting immune-deficiency in HD. This in turn would greatly contribute towards a better understanding of the role of immune-deficiency in the etiopathogenesis of the disease.

INTRODUCTION

ALTHOUGH the occurrence of familial cases of Hodgkin's disease (HD) has been widely reported in the literature for many years [1–10], the significance of HD aggregates in families and communities was investigated particularly during the sixties and seventies.

Cases of HD clustering could be considered as mere medical curiosities or, on the other hand, as having environmental or genetic implications. Vianna and co-workers reported in 1974 an epidemiological study on familial HD [3], suggesting that HD could be interpreted as an environmentally determined disorder in which host reactivity, genetically influenced, may play a role in the various patterns of presentation of the disease.

In recent years, immunological studies performed in HD patients have drawn the attention to the possible role of immune-deficiency (ID) in the etiopathogenesis of HD. As is already known, a characteristic biological feature of HD is an ID involving mainly the cellular response. In untreated patients this impairment is revealed by: (a) a reduced reactivity to delayed hypersensitivity both to recall and to neo-antigens; (b) a decreased number of circulating T lymphocytes; (c) an *in vitro* impaired response to mitogens; (d) a decreased production of interleukin-2; (e) an increased sensitivity to normal suppressor cells [11–20].

Some of these alterations persist in long-term survivors [16–18] and do not appear to be related to the treatment [19]. Moreover, such persistent ID could be in agreement with the occurrence in these patients of secondary non-Hodgkin's lymphomas, of either immunoblastic or Burkitt's type [21, 22].

It is, therefore, still not clear whether the ID in HD should be interpreted as acquired with the disease or as a genetically or environmentally determined feature, pre-existing in patients who will develop HD. In the latter case it would be interesting to detect immunological defects in healthy relatives of HD patients. Björkholm *et al.* reported some alterations of immune functions in healthy twin siblings [23] and in first degree relatives of patients with HD [24]. Nonetheless, these data were not confirmed by others [15]. In this context we thought that an immunological study in individuals belonging to five families with multiple cases of HD could be useful in understanding the role of ID in the etiopathogenesis of the disease.

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MATERIALS AND METHODS

Families

Five families with multiple-case HD were studied at the Hematology Institute of the University 'La Sapienza' of Rome. The familial population consisted of 10 patients (two from each family) with a previous histologically proven HD according to Lukes and Butler [25] and a median age of 27.5 years (range 15–70 years), and of 12 healthy relatives. At the time of the study all patients were in unmaintained complete remission (CR) for a period ranging from 1 to 10 years.

The clinical characteristics of patients and familial pedigrees are reported in Table 1. The group of relatives consists of three parents and nine siblings.

With respect to the group of relatives, the median age was 29.9 years (range 13–41 years). They had all been living together with their respective affected relatives and were in apparent healthy condition when examined, having neither infection nor inflammatory disorder.

Controls

Our control group consisted of 13 age-matched healthy volunteers with a median age of 33.2 years (range 13–45 years).

Monoclonal antibodies (MoAbs)

Peripheral blood mononuclear cells (PBMC) were separated by Ficoll-Hypaque. The reactivity with MoAbs OK T3, OK T11, OK T4 and OK T8 (Ortho Pharmaceuticals, Raritan, NJ) was determined by indirect immunofluorescence as described elsewhere [26].

Mitogen stimulation

Mitogen responses to phytohemagglutinin (PHA, Difco) and concanavalin A (Con-A; Sigma) were assayed on PBMC.

PBMC were washed twice in RPMI 1640, supplemented with antibiotics, glutamine and 20% normal male AB serum; 1×10^5 PBMC suspended in 0.2 ml were cocultured in triplicate in microwells, using the following concentration of mitogens: PHA 10, 20, 30 mmg/ml, Con-A 20 mmg/ml. Cultures were incubated for 72 h at 37°C in 5% CO_2. At 48 h, 2 μCi of [^{3}H]thymidine was added to each well; then the microwells were harvested and counted in a liquid scintillation counter.

Mixed lymphocyte reaction (MLR)

MLR were set up coculturing in triplicate 2×10^5 'responding' lymphocytes and 2×10^5 'stimulating' previously irradiated (2500 r) lymphocytes in a volume of 0.2 ml RPMI 1640 supplemented with antibiotics, glutamine and 20% normal male AB serum. After 5 days of incubation in a humidified 5% CO_2 atmosphere, cultures were pulsed with 2 μCi of [^{3}H]thymidine; and were harvested 24 h later for liquid scintillation counting. For autologous MLR, responding lymphocytes were cocultured with autologous lymphocytes [27].

Sensitivity to suppressor monocytes

The sensitivity of mononuclear cells to monocyte suppression was determined as previously described by Fisher *et al.* [28]. Briefly, one-way MLR were cultured in triplicate utilizing 2×10^5 'responding' cells. Stimulator cells, from one unrelated donor, were first irradiated (2500 r); then 2×10^5 or 8×10^5 stimulating cells were added in each well, in a volume of 0.2 ml RPMI 1640 supplemented with antibiotics, glutamine, and 20% male AB serum. Plates were incubated, pulsed with [^{3}H]thymidine and harvested as described for MLR. The lower proliferation rate observed in the presence of 8×10^5 stimulator cells compared to that observed with 2×10^5 stimulator cells reflects the suppression caused by the increased number of suppressed monocytes [28].

Statistical analysis

Means and standard deviations were calculated. Differences between the groups were evaluated using Student's *t* test.

RESULTS

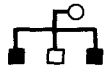
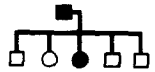
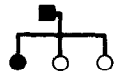
Familial pedigrees together with some epidemiological data and clinical characteristics of the affected members are reported in Table 1. The familial groups included four cases of parent–child affected pairs, and one sibling pair with HD. The mean time interval between diagnosis of affected pairs was 2.6 years (range 0.4–6 years).

The total lymphocyte counts, both in patients and in relatives, did not show significant variations from normal values (2269 ± 721 and 2303 ± 373 in patients and relatives respectively and 1775 ± 369 in normal controls). Therefore, the lymphocyte subsets, as detected with MoAbs, were analysed here considering only the respective percentages.

Figure 1 shows the percentage values of lymphocyte subpopulations detected with MoAbs OKT3, OKT4, OKT8, OKT11, and the T4/T8 ratio. The percentage of T lymphocytes (OKT3+ and OKT11+) was within the normal range in both groups considered.

Regarding the T helper/inducer subsets (OKT4+), significantly decreased percentages were found in both patient and relative groups when compared to normal controls ($P < 0.01$ and < 0.05 respectively). Conversely, the T suppressor/cytotoxic lymphocytes (OKT8+) were significantly increased only in HD patients and conse-

Table 1. Clinical characteristics of patients and their pedigrees

Families	Pedigree	Patient No.	Age (1986)	Age at diagnosis	Histology*	Stage†	Treatment‡	Years without therapy	Time interval between the two diagnoses (mos)	Cohabitation (until diagnosis)
A		1	18	10	MC	IIA	RT + CHT	8	84	Yes
		2	23	22	NS	IIIB	CHT	1		
B		3	70	67	MC	IIB	CHT	2	5	Yes
		4	32	30	LP	IA	RT	2		
C		5	52	41	MC	IA	RT	11	72	Yes
		6	15	9	NS	IA	CHT	5		
D		7	43	34	MC	IIIA	CHT	8	36	Yes
		8	15	9	MC	IVB	CHT + RT	5		
E		9	47	41	NS	IIIB	CHT + RT	5	36	Yes
		10	18	9	NS	IIA	CHT + RT	9		

□ Male; ○ female; ■, ● patients.

*Rye. LP = lymphocyte predominance; MC = mixed cellularity; NS = nodular sclerosis.

†Ann Arbor, 1971.

‡CHT = chemotherapy, MOPP or ABVD; RT = radiotherapy, mantle and inverted Y, plus spleen.

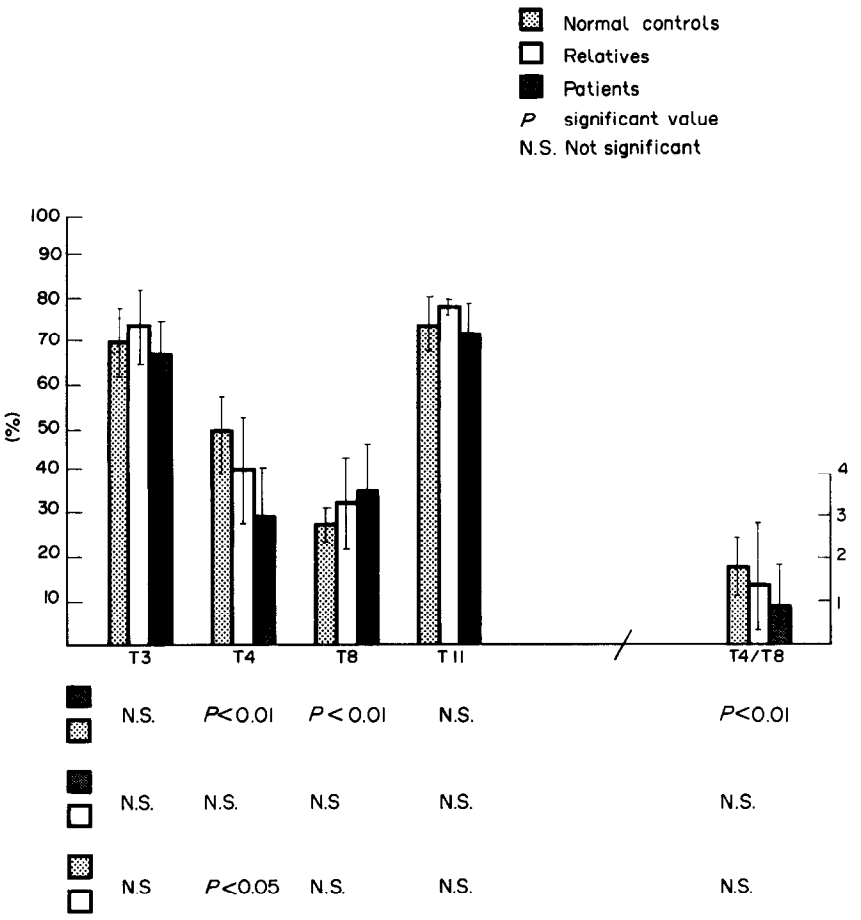


Fig. 1. Percentage values of lymphocytes subsets in Hodgkin's disease patients, relatives and controls.

quently the T4/T8 ratio was significantly ($P < 0.01$) imbalanced only in this latter group.

The results of functional studies are shown in Fig. 2. The PHA-induced lymphoproliferative response was found to be significantly impaired in patients, whatever the concentration employed. In the group of relatives we did not find a statistically remarkable depression of response, whereas in the comparison between patients and relatives a significant impairment was observed at the highest concentration of PHA mitogen. With respect to Con-A, a significantly depressed response was detected in both patients and relatives.

Regarding autologous and allogeneic MLR, no relevant differences were noted and a pattern of activation close to normal values was present also in the patient group.

Finally, the sensitivity to normal suppressor monocytes, though increased in patients and relatives, was not statistically different when compared to normal controls.

DISCUSSION

Our investigation of immunological features in families with multiple cases of HD was carried out because such a population could represent a favourable subject of study to verify the hypothesis of a pre-existing ID in HD.

The hypothesis that ID could play some role in the etiopathogenesis of the disease might be further supported by the evidence of an increased incidence of HD and non-Hodgkin's lymphomas in patients with primitive immunodeficiencies [29]. In particular, HD is more frequently reported in patients affected by ataxia-telcangectasia, a disease in which an imbalance of cellular immune response is also documented [31]. More recently, the occurrence of HD and NHL with an aggressive clinical course was reported in acquired immunocompromised syndromes, such as AIDS and pre-AIDS [30].

Our data confirm the existence of a persisting ID in long-term survivors, characterized by an imbalance of T lymphocyte subsets and a decreased response to the polyclonal mitogens PHA and Con-A. In the patients, neither absolute nor relative T lymphocytopenia was observed and the allogenic MLR was within the lower values of the normal range. These data confirm that after remission there is a progressive recovery of at least some immune functions which were deranged at the onset of the disease and soon after the treatment [15, 18, 31].

In healthy relatives the immunological findings

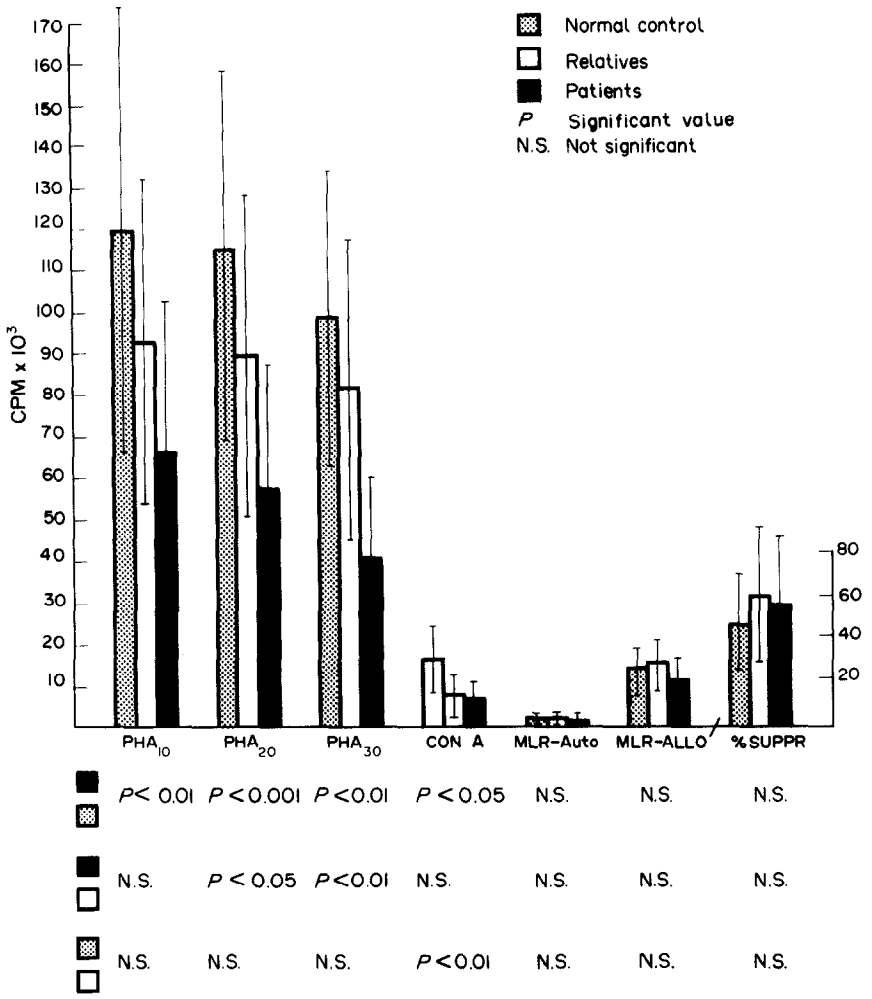


Fig. 2. Lymphocyte response to mitogens (PHA and Con-A), autologous (MLR-Auto) and allogenic (MLR-ALLO) stimuli and sensitivity to normal suppressor monocytes in Hodgkin's disease patients, relatives and controls.

seem to suggest a pattern similar to that of the patients. In particular, a significantly decreased percentage of OKT4+ lymphocytes and a depressed response to Con-A mitogen were detected. It is worth noting that a decreased Con-A stimulation has often been described in other familial HD studies [23, 24, 32]. Moreover, unlike the PHA-induced response, which is not reported to be always significantly impaired, Con-A responsive cells seem to be more often depressed in the persistent ID of HD [23, 24].

The increased sensitivity of T cells to regulation by normal suppressor cells, both to Con-A-induced suppressor cells and to suppressor monocytes, is also reported as persistent in long-term survivors with HD [19].

In this study we have tested the sensitivity of T cells of patients and relatives to normal suppressor monocytes. Though this activity was increased in both instances, the difference was not statistically significant when compared to normal controls.

Our findings seem to favour the hypothesis of the pre-existence of ID in HD. Such a suggestion, indicated by some authors, is still not generally accepted. For instance, Romagnani *et al.* in 74 relatives of HD patients [15] were not able to find statistically significant differences in the mitogen-induced lymphoproliferative responses, nor did they detect quantitative alterations of T and B lymphocytes. In this respect, we believe that a possible explanation of these contradictory results could be found in the sample chosen for this kind of study. For this purpose, the group studied by us can be considered as a high risk sample, in which the chance of detecting the ID is probably higher.

Until today it has not been possible to clarify whether genetic and/or environmental factors are involved in determining the ID in HD. In this respect, no conclusive evidence can be drawn from HLA studies in familial HD. Some interesting observations, such as an excess of HLA identical patients among the pairs of affected siblings have been

reported by Hors *et al.* [9] and by Torres *et al.* [10]. An analysis of HLA types is also planned in our familial groups. The preliminary results obtained by the study of three out of five families do not confirm a similar excess of either identical or aplo-identical HLA sibs (unpublished data).

In conclusion, we believe that the advent of new technology and the extension of genetic studies, together with more accurate epidemiological and immunological studies on 'high risk' families, could provide in the future some useful insight into the understanding of the role of ID in HD.

REFERENCES

1. De Vore JW, Doan CA. Studies in Hodgkin's syndrome. Hereditary and epidemiologic aspects. *Ann Intern Med* 1957, **47**, 300–316.
2. Razis DV, Diamond HD, Craver LF. Familial Hodgkin's disease: its significance and implications. *Ann Intern Med* 1959, **51**, 933–971.
3. Vianna NJ, Davies JNP, Polan AK, Wolfgang P. Familial Hodgkin's disease: an environmental and genetic disorder. *Lancet* 1974, **2**, 854–857.
4. Dworsky RL, Henderson BE. Hodgkin's disease clustering in families and communities. *Cancer Res* 1974, **34**, 1161–1163.
5. Lynch HT, Saldivar VA, Guirgis HA *et al.* Familial Hodgkin's disease and associated cancer. *Cancer* 1976, **38**, 2033–2041.
6. Bowers TK, Moldow CF, Bloomfield CD, Yunis EJ. Familial Hodgkin's disease and the major histocompatibility complex. *Vox Sang* 1977, **33**, 549–554.
7. McBride A, Fennelly JJ. Immunological depletion contributing to familial Hodgkin's disease. *Eur J Cancer* 1977, **13**, 549–554.
8. Greene MH, McKeen EA, Li FP, Blattner WA, Fraumeni JF Jr. HLA antigens in familial Hodgkin's disease. *Int J Cancer* 1979, **23**, 777–780.
9. Hors J, Steinberg G, Andrieu JM *et al.* HLA genotypes in familial Hodgkin's disease. Excess of HLA identical affected sibs. *Eur J Cancer* 1980, **16**, 809–815.
10. Torres A, Martinez F, Gomez P, Gomez C, Garcia JM, Munez-Roldan A. Simultaneous Hodgkin's disease in three siblings with identical HLA genotype. *Cancer* 1980, **46**, 838–843.
11. Holm G, Mellstedt H, Björkholm M *et al.* Lymphocyte abnormalities in untreated patients with Hodgkin's disease. *Cancer* 1976, **37**, 751–761.
12. Björkholm M, Wedelin C, Holm G, Johansson B, Mellstedt H. Longitudinal studies of blood lymphocyte capacity in Hodgkin's disease. *Cancer* 1981, **48**, 2010–2015.
13. Hancock BW, Dunsmore IR, Swan HT. Lymphopenia: a bad prognostic factor in Hodgkin's disease. *Scand J Haematol* 1982, **29**, 193–199.
14. Van Rijswijk REN, Sybesma JHB, Kater L. A prospective study of the changes in the immune status before, during and after multiple-agent chemotherapy for Hodgkin's disease. *Cancer* 1983, **51**, 637–644.
15. Romagnani S, Rossi-Ferrini PL, Ricci M. The immune-derangement in Hodgkin's disease. *Semin Haemat* 1985, *22*, 44–55.
16. Fisher RI, De Vita VT, Bostick F *et al.* Persistent immunological abnormalities in long term survivors of advanced HD. *Ann Intern Med* 1980, **92**, 595–599.
17. Skovmann Sorensen O, Schroder H, Moller Larsen A, Haahr S. Cellular and humoral immunity in Hodgkin's disease. *Scand J Haematol* 1981, **27**, 171–180.
18. Hancock BW, Bruce L, Whetham MD, Dunsmore LR, Milford Ward A, Richmond J. Immunity in Hodgkin's disease: status after 5 years remission. *Br J Cancer* 1982, **46**, 593–600.
19. Vanhaelen CPJ, Fisher RI. Increased sensitivity of T cells to regulation by normal suppressor cells persist in long-term survivors with Hodgkin's disease. *Am J Med* 1982, **72**, 385–390.
20. Ford RJ, Tsao J, Kouttab NM, Sabrasrabuddha CG, Mehta SR. Association of an interleukin abnormality with the T cell defect in Hodgkin's disease. *Blood* 1984, **64**, 386–392.
21. Liechtenstein A, Levine AM, Lukes RJ *et al.* Immunoblastic sarcoma. *Cancer* 1979, **43**, 343–352.
22. Mandelli F, Martelli M, Cimino G *et al.* A case of Burkitt's lymphoma L3 ALL with t(8-14) translocation, developed 10 years after Hodgkin's disease. *Scand J Haematol* 1985, **34**, 97–100.
23. Björkholm M, Holm G, De Faire V, Mellestedt H. Immunological defects in healthy twin siblings to patients with Hodgkin's disease. *Scand J Haematol* 1977, **19**, 396–404.
24. Björkholm M, Holm G, Mellestedt H. Immunological family studies in Hodgkin's disease. *Scand J Haematol* 1978, **20**, 297–305.
25. Lukes RJ, Butler JJ. The pathology and nomenclature of Hodgkin's disease. *Cancer Res* 1966, **26**, 1063–1083.
26. Fontana L, De Rossi G, De Sanctis G *et al.* Decreased NK activity in hairy cell leukemia (HCL). An analysis at the cellular level. *Blut* 1986, **53**, 197–213.
27. Wensler ME, Moody CE Yr, Kozak RW. The autologous mixed-lymphocyte reaction. *Adv Immunol* 1983, **31**, 271–312.

28. Fisher RI, Vanhaelen C, Bostick F. Increased sensitivity to normal adherent suppressor cells in advanced Hodgkin's disease. *Blood* 1981, **57**, 830–835.
29. Frizzera G, Rosai J, Debener LP, Spector BD, Kersey JH. Lymphoreticular disorders in primary immunodeficiencies: new findings based on up-to-date histologic classification of 35 cases. *Cancer* 1980, **46**, 692.
30. Ungerand PD, Stanchen JA. Hodgkin's disease in AIDS complex patients. *Cancer* 1986, **58**, 821–825.
31. Fuks Z, Strober S, Bobrove M *et al.* Long term effects of radiation on T and B lymphocytes in peripheral blood of patients with Hodgkin's disease. *J Clin Invest* 1976, **58**, 803–814.
32. Del Giacco GS, Cengiarotti L, Mantovani G *et al.* Quantitative and functional abnormalities of total T lymphocytes in relatives of patients with Hodgkin's disease. *Eur J Cancer Clin Oncol* 1985, **21**, 795–801.