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Impaired T- and B-cell functions in patients with Hodgkin's disease

Reduced mitogenic responsibility and Il-2 production is not caused by defective CD4$^+$-cells

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Summary. The mitogenic response of T-cell subsets, the production of interleukin-1 (Il-1) and interleukin-2 (Il-2) and in vitro immunoglobulin production was investigated in patients with Hodgkin's disease (HD). The mitogenic response of mononuclear cells (MNC) and the OKT4$^+$ and OKT8$^+$ subsets was greatly reduced in advanced disease stages and could only partially be restored with exogeneous Il-2. In untreated patients with HD – except those with highly advanced disease – the OKT4$^+$ lymphocytes showed normal response to phytohemagglutinin in contrast to the MNC suggesting inhibiting agents or cells within the MNC. These findings corresponded to reduced Il-2 synthesis of MNC, whereas isolated OKT4$^+$ – cells produced normal or elevated amounts of Il-2. MNC or monocytes produced normal or even higher amounts of lipopolysaccharide-induced Il-1 than controls. The results do not confirm a defect in this component of the interleukin system in HD. The immunological impairment was not limited to the T-cell system but involved B-cell activation and differentiation as well. The pokeweed mitogen-induced IgM, IgG and IgG production was highly suppressed in untreated HD, whereas the MNC of previously treated patients produced subnormal amounts of immunoglobulin in vitro. It is not yet clear whether this defect is T-cell-mediated or primarily a B-cell deficiency.

Introduction

Multiple studies have revealed that patients with untreated Hodgkin's disease (HD) had impairments of their cellular immunity, as manifested by impaired delayed hypersensitivity to recall and neoantigens (Advani et al. 1979; Young et al. 1972), decreased lymphocyte and T-cell counts (Bergmann et al. 1985; Björgholm et al. 1981) and reduced responses to lectins or soluble antigens (Schulof et al. 1981; Ziegler et al. 1975). Some of these abnomalities persist even in disease-free long-term survivors (Bergmann et al. 1985; Case et al. 1977). More recently, in vitro studies have focused on regulatory abnormalities of T-cell interaction. Increased suppressor activity of monocytes or T-cells, increased sensitivity of effector T-lymphocytes to normal suppressor cells and circulating soluble suppressor factors have been implicated as causes of the immunological defect (Engleman et al. 1979; Fisher et al. 1981; Hillinger and Herzig 1978). With the development of monoclonal antibodies directed to lymphocyte antigens, it has become possible to characterize and isolate lymphocyte subpopulations with different functional characteristics (Reinherz et al. 1979, 1982; Reinherz and Schlossman 1980). A disequilibrium of peripheral T-cell subsets characterized by monoclonal antibodies has been demonstrated in some patients with HD, particulary in those with constitutional symptoms and/or advanced disease (Bergmann et al. 1985; Björgholm et al. 1982; Schulof et al. 1981). However, the quantitative changes in T-cells and their subsets do not seem to account for the impairment of the in vitro lymphocyte reactions alone (Björgholm et al. 1982). These reactions are mediated by soluble factors produced by macrophages (e.g. interleukin-1 = Il-1) or T-cell subsets (e.g. interleukin-2 = Il-2). These molecules stimulate proliferation and differentitation within the immune system (Falkoff et al. 1983; Farrar et al. 1982; Solbach et al. 1982). To explore the possible defect(s) of the in vitro T-cell reactivity in HD, we studied the phytohemagglutinin (PHA)-induced proliferation of T-lymphocyte subpopulations, the interleukin production of peripheral lymphocytes and monocytes and the pokeweed mitogen (PWM)-induced immunoglobulin (Ig) synthesis by mononuclear cells (MNC) of patients with untreated or previously treated HD.

Materials and methods

Patients

The patient population consisted of 7 newly diagnosed untreated and 7 previously treated patients with HD. All patients with treated disease were in complete remission at the time of investigation. The main clinical characteristics and some immunological findings are summarized in Table 1. Peripheral blood was taken from the untreated patients at the time of initial presentation, before clinical and staging procedures were instituted. Age-matched healthy volunteers were used as controls. Clinical and pathological stage were defined according to the Ann Arbor classification. In the treated group, patients had received radiotherapy or combined modality treatment (Table 1). Chemotherapy consisted of cyclophosphamide, vincristine, procarbazine and prednisolone.

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Table 1. Patient characteristics

Patient	Age/sex	Stage	Histology	Pre-treatment	Therapy-free intervall (months)	Lymphocytes	OKT3+	OKT4+	OKT8+	OKT4/OKT8
						Cells/μl				
1	37 F	IA	NS	no	–	1197	886	622	287	2.17
2	20 M	IIA	NS	no	–	920	644	258	377	0.68
3	23 M	IIA	LP	no	–	2680	2064	1501	670	2.24
4	26 M	IIB_S	MC	no	–	2550	1836	1148	714	1.61
5	25 M	IIIB	MC	no	–	1824	1058	584	474	1.23
6	68 M	IIIB	MC	no	–	n.d.	n.d.	n.d.	n.d.	n.d.
7	38 M	IVB	MC	no	–	493	375	316	74	4.27
8	23 F	IA	MC	RT	35	1980	1287	673	594	1.13
9	40 F	IIA	NS	RT	39	1771	797	266	531	0.50
10	44 F	IIIA	MC	CT, RT	82	1976	1146	692	514	1.35
11	34 M	IIIA	LP	CT, RT	12	1704	699	341	341	1.00
12	46 F	IIIB	NS	CT, RT	36	3337	1835	734	1168	0.62
13	29 M	IVB	LP	CT, RT	12	2204	1212	507	639	0.79
14	45 F	IVB	MC		30	4290	3046	1888	1244	1.52
Normal values						1933 ± 577	1527 ± 403	1030 ± 317	507 ± 136	2.07 ± 0.51

LP = lymphocytic predominant; NS = nodular sclerosis; MC = mixed cellularity; CT = chemotherapy; RT = radiotherapy; n.d. = not determined

Methods

Lymphocyte separation. The MNC were separated by Ficoll-Hypaque sedimentation from heparinized peripheral blood. The viability determined by trypan blue exclusion as was approximately 98%.

Surface markers. The methods for surface marker studies have been described in detail elswhere (Bergmann et al. 1982). In brief, surface Ig-expressing cells (sIg, B-cells) were identified by an immunofluorescent test using luorescein isothiocynate (FITC)-labelled goat-anti-human-Ig F(ab) fragments (Kallestadt Lab., Chaska, Minn.). T-cells and T-cell subsets were marked with monoclonal antibodies OKT3, OKT4, OKT8; Ortho, Westwood, Mass.; Reinherz and Schlossman 1980) using FITC-labelled goat anti-mouse-Ig F(ab) fragments as second antibodies (Tago, Burlingame, Calif.). A minimum of 200 cells was evaluated using a Zeiss microscope equipped with vertical fluorescent illumination and phase contrast. The absolute counts of lymphocyte subsets were calculated from their proportion of the total lymphocyte count.

Isolation of macrophages and T-cell subsets. Macrophages were obtained from MNC by adherence to plastic according to Morimoto et al. (1981). The adherent cells (AC) were finally resuspended at a concentration of 0.25×10^6 cells/ml. More than 85% of the AC had morphological characteristics of monocytes by Pappenheim staining and were capable of ingesting latex particles.

For functional analyses T-cell subsets were separated by a modified panning technique according to Reinherz et al. (1979). The 6-well macrotitre plastic plates (Greiner, Nürtingen, FRG) were coated with 1 μg/well of affinity purified goat-anti-mouse-Ig (Tago, Burlingame, UK) at 4 °C for at least 18 h prior to use. Then the plates were washed with cold phosphate-buffered saline solution (PBSS) and filled with PBSS supplemented with 2% fetal calf serum (FCS) and 1% penicillin-streptomycin until addition of labelled cells. Concurrently, the non-adherent cells were subdivided and marked either with OKT4 or OKT8. After 3 washings with RPMI 1640 the pellet was resuspended in RPMI 1640 at a concentration of 6×10^6 cells/ml. Then 1 ml of suspension was added to each coated well, centrifuged at 100 *g* for 5 min and incubated for 30 min at 37 °C. After removing the supernatant by extensive washings the adherent lymphocytes were removed after an incubation with RPMI 1640 containing 2.5 m*M* EDTA for 1 h at 37 °C and resuspended in RPMI 1640 at 3×10^6 cells/ml. The positively separated cells were enriched to about 95% as tested by immunofluorescent staining.

Proliferation assays. The MNC, OKT4+ and OKT8+ lymphocytes were tested for their mitogenic response to PHA with or without substitution of 10% human Il-2 (Lymphocult-T, Biotest, Frankfurt, FRG) which has been shown to be the optimal concentration in controls and patients (data not shown). The microcultures with 50000 cells/well were performed in triplicate at optimal doses of 0.5 μg/ml PHA-P (Wellcome, Beckenham, UK). To the separated OKT4+ and OKT8+ cells, 10% macrophages were added. The cells were cultured for 72 h at 37 °C in a humidified atmosphere and afterwards pulsed with 0.5 μCi/well H^3 thymidine. The H^3 thymidine uptake was measured after 24 h. All cultures were performed with RPMI 1640 supplemented with 10% FCS, 2 m*M* L-glutamine, 10 m*M* Hepes buffer and 1% penicillin-streptomycin.

Il-2 production and Il-2 assay. A 1.5×10^6 cells/ml sample of MNC or OKT4+ lymphocytes was stimulated with 0.5 μg/ml PHA-P for 24 h at 37 °C. Afterwards, the supernatant was removed and stored at −20 °C until assayed for Il-2 activity. For the Il-2 assay, 5000 murine Il-2-dependent cytotoxic T-lymphocytes were grown in the presence of log dilutions of the putative Il-2-containing supernatant in microtitre plates (Nunc, Kampstrup, Denmark) in a total volume of 200 μl/well for 28 h. At 6 h prior to harvesting, the microcultures were pulsed with 0.5 μCi H^3 thymidine. The Il-2 concentrations were calculated by

probit analysis using standard Il-2 preparations (Lymphocult-T, Biotest, Frankfurt, FRG).

Il-1 production and Il-1 assay. A 2.5×10^6 cells/ml sample of MNC or monocytes was stimulated with 20 µg/ml lipopolysaccharide (LPS) *(Escherichia coli* 055:B5, Difco, Detroit, Mich.) for 24 h at 37 °C and the putative Il-1-containing supernatants removed. The samples were assayed for Il-1 activity using C3H/HeJ mice thymocytes according to Falkoff et al. (1983). Thymocytes were incubated in microplates with 0.5×10^6 cells/well in 100 µl fully supplemented RPMI 1640 in the presence of 0.5 µg PHA-P and $\log_2$ dilutions of putative Il-1-containing supernatants (100 µl/well) for 96 h at 37 °C. The cultures were pulsed with 0.5 µCi/well H^3 thymidine 24 h before harvesting. The Il-1 concentrations were calculated by probit analysis using the Il-1-containing supernatant of an acute myelomonocytic leukemia (M4-FAB) as in-house laboratory standard.

In vitro Ig synthesis. Peripheral MNC were tested for their ability to synthesize Ig in vitro spontaneously or after PWM-induced stimulation. Using a total volume of 200 µl 0.2×10^6 cells/well were cultured with or without 1.0 µg/ml PWM (Gibco, Long Island, NY) at the optimal dilution for 7 days at 37 °C. The supernatants were removed and tested for IgA, IgG and IgM by a modified enzyme-linked immunosorbent assay (ELISA) technique with AP-labelled antibodies according to Engval and Perlman (1971). The sensitivity was 25 ng/ml.

Statistics. Differences between groups were tested for statistical significance by the *t*-test for dependent samples.

Results

Mitogen stimulation of MNC and T-cell subsets

The MNC and T-cell subsets were cultured with PHA alone or PHA and varying dilutions of partially purified Il-2. A 10% (vol/vol) preparation of Il-2 was the optimal final concentration for increasing the lymphocyte response to PHA in controls and patients.

Table 3. PHA-induced Il-2 production of MNC or separated OKT4+ cells and lipopolysaccharide-induced Il-1 production of MNC and monocytes in patients with Hodgkin's disease (HD)

Il-2 Production (units/ml[a])	MNC	OKT4+ cells
Controls	1.2 ± 1.0	4.6 ± 5.3
HD, untreated	0.6 ± 0.7	6.0 ± 6.8
Il-1 Production (units/ml[a])	**MNC**	**Monocytes**
Controls	0.9 ± 0.6	1.0 ± 0.6
HD, untreated	2.2 ± 1.7	2.6 ± 1.1

[a] Definition of units see *Materials and methods*

The proliferative response of the MNC of 4 untreated patients (patients 1, 2, 4, 5) was low but still within the normal range (Table 2). Isolated $OKT4^+$ and $OKT8^+$ lymphocytes showed a normal response to PHA in 5 cases (patients 1–5). In the 2 cases with depressed proliferative response of both $OKT4^+$ and $OKT8^+$ cells to PHA (patients 6, 7) exogenous Il-2 increased the proliferation of the $OKT4^+$ lymphocytes, but did not restore the mitogenic response (Table 2).

Similar results were obtained with MNC and separated T-cell subsets of patients in remission (Table 2). In 1 of 3 cases (patient 10) the Il-2 substitution normalized the depressed proliferative response of the MNC. Stimulation of MNC, $OKT4^+$ and $OKT8^+$ cells was subnormal or decreased with and without addition of Il-2 in the remaining 2 cases (patients 13, 14) with stage IV disease prior to treatment (Table 2).

Lymphokine synthesis

LPS-stimulated MNC or peripheral monocytes of patients with untreated or previously treated HD produced higher amounts of Il-1 when compared to the controls (Table 3). Production of Il-2 was only studied in patients with untreated HD. Their MNC synthesized less Il-2, whereas separated $OKT4^+$ cells were able to produce comparable

Table 2. Phytohemagglutinin (PHA)-induced proliferation of mononuclear cells (MNC) and T-cell subsets with and without substitution of human interleukin-2 (Il-2) (cpm × 10^3)

Patient	MNC		OKT4+-cells		OKT8+-cells	
	− Il-2	+ Il-2	− Il-2	+ Il-2	− Il-2	+ Il-2
1	12.0	22.1	38.1	48.3	18.5	30.7
2	17.7	19.1	28.5	25.2	25.2	18.7
3	5.1	21.1	14.8	30.5	13.3	17.4
4	15.4	34.3	59.7	93.9	48.3	35.9
5	12.1	12.9	54.1	63.4	12.5	13.9
6	3.8	4.2	4.0	10.4	4.5	4.6
7	2.9	5.6	1.1	2.2	0.5	1.1
8	29.2	32.7	29.6	32.6	25.0	26.7
9	–	–	–	–	–	–
10	9.8	24.0	21.7	28.0	14.8	16.4
11	17.2	19.8	11.6	17.8	14.8	13.8
12	13.9	17.9	13.8	14.6	9.8	10.5
13	2.5	3.2	1.9	2.9	1.5	1.9
14	10.6	10.7	5.9	6.5	1.6	2.5
Controls	34.1 ± 21.1	34.5 ± 15.5	35.8 ± 21.5	44.4 ± 25.3	23.8 ± 15.2	26.7 ± 12.9

Table 4. Spontaneous and pokeweed mitogen (PWM)-induced immunoglobulin (Ig) production of MNC in patients

Patient	PWM-Ig induced synthesis						Serum		
	IgM (ng/ml)		IgG (ng/ml)		IgA (ng/ml)		IgM mg%	IgG mg%	IgA mg%
	−PWM	+PWM	−PWM	+PWM	−PWM	+PWM			
1	102	305	460	630	304	310	238	1010	431
2	40	115	455	350	60	80	78	1580	385
3	160	2410	270	2070	75	710	66	1120	197
4	<30	215	300	340	320	385	243	831	163
5	55	75	190	450	205	170	402	1710	332
6	2970	1940	–	2270	470	305	816	1150	173
7	<30	40	350	350	–	455	47	433	52
Median	55*	215*	325	450*	255	310	238	1150	197
range	(<30–2,970)	(40–2,410)	(190–460)	(350–2,270)	(75–470)	(80–710)	(47–816)	(433–710)	(52–431)
8	185	1650	440	2655	240	540	138	1230	276
9	<30	455	600	1380	220	460	63	947	220
10	<30	4700	550	15630	180	2305	53	1590	282
11	<30	880	415	7530	245	900	56	980	134
12	–	–	–	–	–	–	57	1430	227
13	115	890	615	920	360	335	77	983	99
14	<30	555	210	715	120	315	64	1120	172
Median	<30*	885	485	2020	230	500	63*	1120	220
range	(<30–185)	(455–4,700)	(210–615)	(715–15,630)	(120–360)	(315–2,305)	(53–138)	(947–1,590)	(99–282)
Controls									
Median	400	2430	650	2605	260	1055	122	1117	209
range	(<30–1,405)	(790–7,585)	(180–3,355)	(1,720–4,800)	(<30–1,300)	(360–2,325)	(71–386)	(879–1,660)	(133–299)

* $P < 0.05$ compared to controls

amounts of Il-2 when compared to healthy individuals (Table 3).

Ig synthesis in vitro

In vitro Ig synthesis was measured using an ELISA technique after a 7-day culture of MNC with or without the T-cell-mediated polyclonal B-cell activator PWM. MNC from untreated and previously treated patients spontaneously (without PWM) produced small amounts of all three Ig classes (Table 4). Spontaneous IgM synthesis was increased in only 1 untreated case (patient 6) corresponding to elevated polyclonal IgM levels in the serum.

PWM-stimulated MNC produced very low levels of IgG and IgM in 5 out of 7 patients with untreated HD. In the case (patient 6) with a high spontaneous IgM synthesis, B-cell stimulation with PWM did not further increase Ig production. In 3 out of 7 cultures, IgA synthesis was comparable to that of the controls (Fig. 1). In previously treated patients with HD the PWM-induced Ig production varied considerably and did not differ significantly from that of the controls (Table 4, Fig. 1).

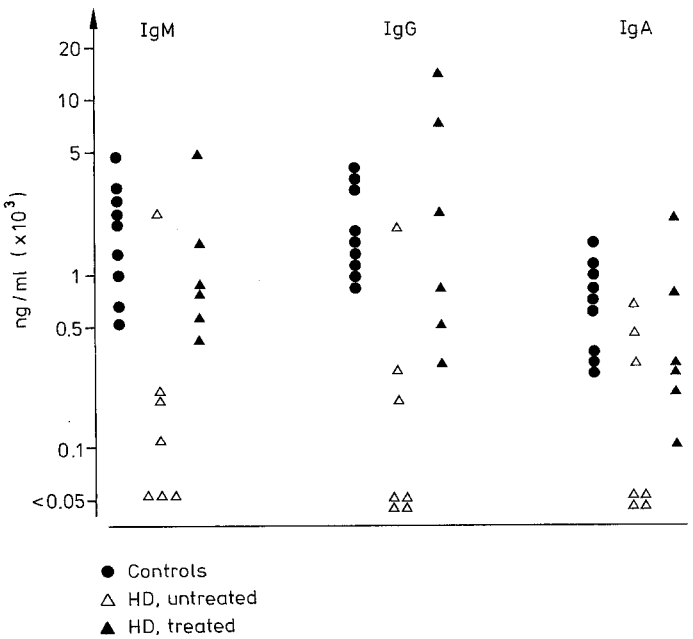

Fig. 1. PWM induced 1 g synthesis in HD

Discussion

The immunological abnormalities in HD are reflected by impaired cell-mediated immunity including depressed proliferative responses of T-cells to mitogens and antigens in vitro. Recent studies have demonstrated that imbalances of T-cell subsets may occur in the peripheral blood and between blood and lymphatic tissues in patients with HD (Bergmann et al. 1985; Forni et al. 1985; Schulof et al. 1981). The exact aetiology of the T-cell defects are still obscure. Our in vitro studies suggest that not only one but different mechanisms may be responsible for the abnormalities detected.

In accordance with other investigators (Schulof et al. 1981; Ziegler et al. 1975) the proliferative response of MNC to PHA was decreased in all patients with untreated HD irrespective to the stage of disease. In contrast isolated T-cell subsets had a normal mitogen-induced DNA synthesis in the majority of these patients. These results sup-

port the idea that the isolation procedures of the T-cell subsets may eliminate soluble or cellular suppressive agents in the MNC fraction and enable a normal response of T-cell subsets to PHA. Recombination experiments between various lymphocyte-monocycyte fractions from the same patient and from patients and healthy donors are required to confirm this hypothesis. However with regard to suppressor mechanisms as a cause for the impaired immune response in HD, earlier studies have demonstrated that T-cell and/or macrophage-mediated suppression account for a considerable degree of the immunodeficiencies. Both increased suppressor activities of monocytes and T-cells and an increased sensitivity of effector cells to suppressor cells have been described (Case et al. 1977; Fisher et al. 1981; Hillinger et al. 1978; Vanhaelen et al. 1981). Furthermore, monocytes in HD have been reported to produce high levels of prostaglandin E (PGE), which is known to suppress Il-2 production and may account for immunological impairment (Rappaport and Dodge 1982; Estevez et al. 1985).

One cause for the depressed mitogenic response of MNC in HD may be the highly decreased Il-2 production of MNC (Table 3, Ford et al. 1984). However, isolated $OKT4^+$ lymphocytes produced amounts of Il-2 comparable to those of the controls (Table 3). These results suggest that the relative inability of MNC to generate Il-2 activity after mitogenic stimulation is not a primary cellular defect of the predominantly Il-2-producing T-cell population as discussed in earlier reports (Ford et al. 1984). Additionally, an abnormality of the expression of the Il-2 receptor does not appear to play a major role in the proliferative impairment of T-cells, as exogenous Il-2 normalized or at least partially restored the response to PHA in most untreated patients.

In 4 untreated or previously treated patients with advanced disease stages and constitutional symptoms the proliferative response of MNC and separated $OKT4^+$ and $OKT8^+$ lymphocytes was markedly suppressed. Exogenous Il-2 was not able to restore the mitogenic response. These data may indicate an intrinsic defect of MNC and T-cell subsets in this subgroup of patients and exclude the possibility of a lack of Il-2 production as the major site of hyporesponsiveness to PHA within this group. Additionally, suppressive mechanisms may be responsible for the reduced proliferative response of T-cell subsets in the patients with advanced stage of disease, including an increased susceptibility of the proliferating cells to normal suppressor cells or an increased suppressive activity in each T-cell subset. The latter possibility should also be considered, as the $OKT4^+$ lymphocytes are heterogeneous and contain a subset of suppressor-inducer cells (Reinherz et al. 1982; Thomas et al. 1981).

In vitro substitution of Il-2 increased the mitogenic response of $OKT4^+$ cells to PHA in healthy controls ($P<0.05$) and in some patients with HD. In contrast, the proliferation of $OKT8^+$ cells was not changed by the addition of Il-2 in controls and patients. It is conceivable that pre-incubation of T-cells with the OKT8 antibody may inhibit the mitogenic response of $OKT8^+$ cells and provides a negative response to Il-2 (Welte et al. 1983).

The importance of the monokine Il-2 in the scheme of T-cell activation has been previously demonstrated (Falkoff et al. 1983; Kaye et al. 1984). Il-2 initiates T-cell activation after antigenic recognition and induces the production and secretion of Il-2 and the expression of Il-2 receptors (Kaye et al. 1984). To ascertain if patients with HD have a defect in this part of T-cell activation, we studied the LPS-induced Il-1 production in these patients. MNC or monocytes from untreated patients produced comparable or even higher amounts of Il-1 than the corresponding cells from healthy controls. These results and those of earlier reports (Ford et al. 1984) do not confirm a defect in this component of the interleukin system in HD. However, it remains to be delineated, whether the recently described hypersecretion of PGE by monocytes in HD plays a major role in the immunodeficiency of these patients (Estevez et al. 1985).

It is assumed that humoral immunoreactions are not impaired in HD. Surprisingly, the PWM-induced Ig synthesis was reduced or even absent in the majority of patients with untreated HD. MNC from patients in remission produced varying amounts of Ig in vitro (Fig. 1). There was considerable overlap between this patient group and the controls with regard to individual responses to PWM. The activation of B-cells by PWM is a complex event requiring a number of antigen non-specific growth and maturation factors produced by activated macrophages and 'helper' T-cells (Howard et al. 1984). In addition, PWM activates precursors of suppressor cells inhibiting Ig synthesis (Miyawaki et al. 1982). Depressed PWM-induced Ig synthesis could be due to a primary defect of B-cell promoting factors or to excessive suppressor cell activity. The normalization of in vitro Ig production in disease-free survivors suggests a reversible defect of T-B interaction.

The data presented reveal heterogeneity of the mechanism(s) accounting for the immune dysfunctions in HD, which is assumed to be dependent on the stage of disease and therapy. Both, T- and B-cells are involved, whereby the major defect may be located within the T-cell system. The elucidation of the aetiology of the immunological impairments in HD may open the possibility for immune-restoring therapeutic modalities.

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References

Advani SH, D'Silva H, Gothoskar BP, Dinshaw KA, Nair NC, Gopalkrishna DR, Talwakar GV, Desai PB (1979) Cellular immunity in Hodgkin's disease. Cancer 43: 492

Bergmann L, Mitrou PS, Cherdron T, Holloway K, Jost J, Timm V, Zerth G (1982) Expression of Ia-like antigen in lymphatic leukemias and non-Hodgkin's lymphomas in correlation with other surface markers. Scand J Haematol 29: 224

Bergmann L, Mitrou PS, Kelker W, Weber KC (1985) T-cell subsets in malignant lymphomas and monoclonal gammopathies. Scand J Haematol 34: 170

Björgholm M, Wendelin C, Holm G, Johansson B, Mellstedt H (1981) Longitudinal studies of blood lymphocyte capacity in Hodkin's disease. Cancer 48: 2010

Björgholm M, Wendelin C, Holm G, Ogenstadt S, Johansson B, Mellstedt H (1982) Immune status of untreated patients with Hodgkin's disease and prognosis. Cancer Treat Rep 66: 701

Case DC, Hansen JA, Corrales E, Young CW, Dupont B, Pinsky CM, Good RA (1977) Depressed in vitro lymphocyte re-

sponses to PHA in patients with Hodgkin's disease in continuous long remissions. Blood 49: 771
Engleman EG, Benike C, Hoppe RT, Kaplan HS (1979) Suppressor cells of the mixed lymphocyte reaction in patients with Hodgkin's disease. Transplant Proc 11: 1827
Engval E, Perlaman P (1971) Enzyme-linked immunoabsorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochemistry 8: 874
Estevez ME, Ballart IJ, Diez RA, Sen L, Nicastro MA, Wechsler A (1985) Normalization of monocyte candidacidal deficiency by cyclooxygenase inhibitors in Hodgkin's disease. Cancer 55: 2774
Falkoff RJM, Muraguchi A, Hong J, Buller JL, Dinarillo CA, Fauci AS (1983) The effects of interleukin 1 on human B cell activation and proliferation. J Immunol 131: 801
Farrar RJM, Benjamin WR, Hilfiker ML, Howard M, Farr WL, Fuller-Farrer J (1982) The biochemistry, biology and role of interleukin 2 in the induction of cytotoxic T cell antibody forming B cell responses. Immunol Rev 63: 129
Fisher RI, Vanhaelen C, Bostick F (1981) Increased sensitivity to normal adherent suppressor cells in untreated advanced Hodgkin's disease. Blood 57: 830
Ford RJ, Tsao J, Kouttab NM, Saharabuddhe CG, Metha SR (1984) Association of interleukin abnormality with the T-cell defect in Hodgkin's disease. Cancer 55: 728
Forni M, Hofman FM, Parker JW, Lukes RJ, Taylor CR (1985) B- and T-lymphocytes in Hodgkin's disease. Cancer 55: 728
Hillinger SM, Herzig GP (1978) Impaired cell mediated immunity in Hodgkin's disease mediated by suppressor lymphocytes and monocytes. J Clin Invest 61: 1620
Howard MK, Nakanishi K, Paul WE (1984) B cell growth and differentiation factors. Immunol Rev 78: 185
Kaye J, Gillis S, Mizel SB, Shevach EM, Malek TR, Dinarello CA, Lachmann LB, Janeway CA (1984) Growth of a cloned helper T cell line induced by monoclonal antibody specific for the antigen receptor interleukin 1 is required for the expression of receptors for interleukin 2. J Immunol 133: 1339
Miyawaki T, Nagaoki T, Yokoi T, Yachie A, Uwadana N, Taniguchi N (1982) Cellular interactions of human T cell subsets defined by monoclonal antibodies in regulating B cell differentiation: A comparative study in Nocardia water-soluble mitogen and pokeweed mitogen-stimulated culture systems. J Immunol 128: 899
Morimoto C, Todd RF, Distaso JA, Schlossman SF (1981) The role of the macrophage in vitro primary anti-DNP antibody production of human interleukin 2. J Exp Med 155: 943
Rappaport RS, Dodge GR (1982) Prostaglandin E inhibits the production of human interleukin 2. J Exp Med 155: 943
Reinherz EL, Kung PC, Goldstein G, Schlossman SF (1979) Separation of functional subsets of human T cells by a monoclonal antibody. Proc Natl Acad Sci 76: 4061
Reinherz EL, Schlossman SF (1980) Regulation of the immune response. Inducer and suppressor T-lymphocyte subsets in human beings. N Engl J Med 303: 370
Reinherz EL, Morimoto C, Fitzgerald KA, Hussey RE, Daley JF, Schlossman SF (1982) Heterogeneity of human T4 inducer cells defined by monoclonal antibody that delineates two functional subpopulations. J Immunol 128: 463
Schulof RS, Lacher MJ, Gupta D (1981) Abnormal phytohemagglutinin-induced T-cell proliferative response in Hodgkin's disease. Blood 57: 607
Solbach W, Barth S, Röllinghoff M, Wagner H (1982) Interactions of human T cell subsets during the induction of cytotoxic T lymphocytes: the role of interleukins. Clin Exp Immunol 49: 167
Thomas Y, Rogozinsky L, Irigoyen OH, Friedman SM, Kung PC, Goldstein G, Chess L (1981) Functional subsets of human T cell subsets defined by monoclonal antibodies. Induction of suppressor cells within the OKT4 population. J Exp Med 154: 459
Welte K, Venuta S, Wang CY, Feldman SP, Ciobanu N, Kruger G, Feickert HJ, Merluzzi VJ, Flomenberg N, Moore MAS, Mertelsmann R (1983) Human interleukin 2: Physiology, biochemistry, and pathophysiology in lymphoblastic leukemias and immunodeficiency syndromes. Haematol Blood Trans 28: 369
Young RC, Corder MP, I aynes HA, DeVita VZT (1972) Delayed hypersensitivity in Hodgkin's disease. A study of 103 untreated patients. Am J Med 52: 63
Ziegler JB, Hansen P, Penny R (1975) Intrinsic defect in Hodgkin's disease: Analysis of the phytohemagglutinin dose response. Clin Immunol Immunopathol 3: 451

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