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Immunological abnormalities in intravenous drug abusers and relationship to the prolonged generalized lymphadenopathy syndrome in Italy

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SUMMARY

The prolonged generalized lymphadenopathy syndrome (PGL) has been considered a prodromal condition to the Acquired immunodeficiency syndrome (AIDS), but the clinical, virological and immunological characteristics of patients who will develop AIDS are not known. We report on the immunological profile of intravenous drug abusers with or without PGL in Northeastern Italy. We found a reduction of lymphocyte-absolute numbers with reversal of the T4/T8 ratio and decreased Leu-11b$^+$ cells. The response to mitogens and natural killer activity are compromised in PGL patients. Neutrophil function is reduced both in drug abusers with or without lymphadenopathy. The serological investigations revealed a high prevalence of antibodies against HTLV III and the Epstein–Barr viruses.

The recognition of immune dysfunction in the intravenous drug abusers appears to be important since these patients develop AIDS and these abnormalities may precede AIDS.

Keywords drug abusers HTLV III lymphocytes lymphadenopathy

INTRODUCTION

The acquired immunodeficiency syndrome (AIDS) is characterized by various clinical, epidemiology and immunological features (Gottlieb *et al.*, 1981; Masur *et al.*, 1981; Siegal *et al.*, 1981; Seligmann *et al.*, 1984). Along with this well defined disease, a variety of heterogeneous symptoms like the prolonged generalized lymphadenopathy (PGL) have been recently reported (Mildvan *et al.*, 1982; Metroka *et al.*, 1983; Enlow *et al.*, 1983). However the possible relationships between AIDS and PGL are not completely understood, although some data suggest that PGL may be a prodromal phase of AIDS.

Since AIDS was first recognized, it has become clear that both immunosuppressive factors and transmissible agents are involved in the pathogenesis of the disease. However the immunological characteristics of those who will develop AIDS remains to be established. This syndrome, initially reported in high risk areas of the United States, affects some distinct groups, like homosexuals, intravenous drug abusers and haemophiliacs (Pinching, 1984), although there is increasing evidence of disease in subjects with no apparent risk factors.

In Europe the earliest rise has occurred in France and in the United Kingdom. Only sporadic cases have been reported in Italy, despite the presence of anti-HTLV-III antibodies in many patients at risk for AIDS (Aiuti, 1984; Rossi *et al.*, 1984).

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Therefore, we have evaluated the clinical and immunological parameters in Italian intravenous drug abusers with or without PGL. No AIDS cases have been reported in our region so the data obtained in our study should be useful in future epidemiological evaluations.

MATERIALS AND METHODS

Patients. Seventy patients, from a geography restricted area of north east Italy, were referred to us between May and December 1984 (15 females & 55 males, mean age 25 ± 6 years). The requirements for entry in the study were regular intravenous (i.v.) heroin use for at least 6 months and heterosexual behaviour. Twenty subjects (7 females & 13 males) had PGL: two or more extrainguinal sites being involved, the presence of fever or nocturnal sweats lasting at least 3 months, lymphopaenia and polyclonal hypergammaglobulinaemia. None of these patients was grossly underweight. Repeated evaluations, including latex agglutination test for infectious mononucleosis, immunofluorescence for *Toxoplasma gondii* antibodies and lymph node biopsy failed to reveal known causes of lymphadenopathy. Hepatic function was normal (serum transaminase levels were periodically measured).

The controls were 20 blood donors of the same age group.

Methods. Peripheral blood anticoagulated with heparin (10 U/ml) was sedimented on Plasmagel for 30 min at 37°C. The leucocyte rich buffy coat was used to quantitate lymphocyte subsets with monoclonal antibodies. Briefly 100 μl of buffy coat were incubated with each monoclonal antibody for 30 min at 4°C. Three washings with phosphate buffered saline (PBS) were performed and a goat antimouse antiserum, FITC conjugated and affinity purified on Sepharose 4B and human Ig column, was added (30 min at 4°C). After three washings, the fluorescence was measured on a Spectrum III cytofluorograf (De Paoli *et al.*, 1984). For mitogen responsiveness, leucocytes were layered on Ficoll Paque and the mononuclear cells obtained stimulated for 3 days, using a microculture technique, by optimal doses of phytohaemagglutinin (PHA) (Difco, Detroit, MI, USA), Concanavalin A (Con A) (Miles Yeda, Kankakee, IL, USA) and pokeweed mitogen (PWM) (GIBCO, Grand Island, NY, USA). The proliferative response was measured by ^{3}H-thymidine incorporation (specific activity 11 Ci/mmole). Natural killer activity was investigated by measuring ^{51}Cr release on K 562 cells after 18 h incubation, following plastic adherence to eliminate monocytes.

Polymorphonuclear leucocytes (PMN) were obtained from the fraction at the bottom of the tubes after Ficoll Paque centrifugation. For the chemiluminescence assay, 2×10^{5} cells in Ca^{2+} Mg^{2+} PBS, 2×10^{-5} M Luminol (Lumac, Titusville, USA) and opsonized zymosan (1 mg/ml) were mixed in the counting chamber of a Picolite Packard luminometer at 37°C. Control samples were run simultaneously. Results are expressed as Relative Light Units (RLUs). Phagocytosis was measured by FITC conjugated latex beads (1·7 μ diameter). After 30 min incubation at 37°C with PMN in the presence of pooled serum, phagocytozing cells were quantified on a Spectrum III cytofluorograf with the trigger region set on the PMN cluster and fluorescence (cells alone) was below 1%. Complement fixing antibodies against Herpes simplex and Herpes zoster viruses and Cytomegalovirus were also determined (Lennette, Melnick & Jahlberg, 1980). IgM and IgG titres against Epstein–Barr virus (EBV) were measured by immunofluorescence on the HRK cell line.

Antibodies to HTLV-III were measured by ELISA (Abbott Diagnostics, Chicago, IL, USA). Positive (human plasma positive at minimum 1:2 titre) and negative controls were run at the same time. The cut-off value was calculated by the following formula: negative control absorbance- $+(0{\cdot}1 \times$ positive control absorbance). Specimens whose values were greater than the cut-off value were considered reactive and retested: specimens which were found repeatedly reactive were interpreted to be positive for anti HTLV-III antibodies. Circulating immune complexes (CIC) were measured by the Bovine Conglutinine technique (ELISA, CliniCals.C.Erba, Milan).

RESULTS

Two important differences in lymphocyte subsets were seen in the i.v. drug abusers compared to the

Table 1. Percentage and absolute numbers/μl ($\pm$s.d.) of the lymphocyte subpopulations recognized by various monoclonal antibodies in drug abusers with or without PGL

		OKT11	OKT3	OKT4	OKT8	HLA-DR	B1	SIgD	Leu-7	Leu-11b	T4/T8
Controls ($n=20$)	Percentages	70±10	69±5	36±4	30·4±5	14±3	6±3	7±3	15±8	13±3	1·4±0·5
	Absolute numbers		1420±340	840±200	610±250					265±60	
Drug abusers	Percentages	68·7±8	64·5±9	34·1±9	32·6±9	13·4±4	8·8±3	6±3	16·6±6	9·9±6	1·1±0·6
without PGL ($n=50$)	Absolute numbers		1210±350	630±180	585±210				160±95*		
PGL patients ($n=20$)	Percentages	71·7±8	76·4±6	35·1±9	39·9±9*	10·7±3	8·1±3	7±3	22·9±7*	7·7±6*	0·86±0·4*
	Absolute numbers		910±80†	405±45*	455±60*					130±90*	

* $P<0·01$.
† $P<0·001$.

healthy controls (Table 1). The OKT8$^+$ cells were increased and the OKT4$^+$ cells decreased with a significant reduction of the T4/T8 ratio ($P < 0{\cdot}01$); the percentages of total T and B lymphocytes were not modified. Natural killer cells, recognized by Leu-11b anti IgG Fc receptor monoclonal antibody, were reduced particularly in the PGL subjects ($P < 0{\cdot}01$). We found a small subset of circulating lymphocytes with transferrin receptors (OKT9$^+$) and by using double fluorescence we identified a small subset of HLA-DR$^+$, interleukin 2 (IL-2) receptor negative T cells (data not shown). The absolute number of lymphocyte subpopulations (Table 1) was reduced, this reduction being more pronounced for the OKT4$^+$ and Leu-11b$^+$ cells in the PGL patients.

Figure 1 shows the proliferative response to PHA, Con A and PWM in drug abusers, PGL

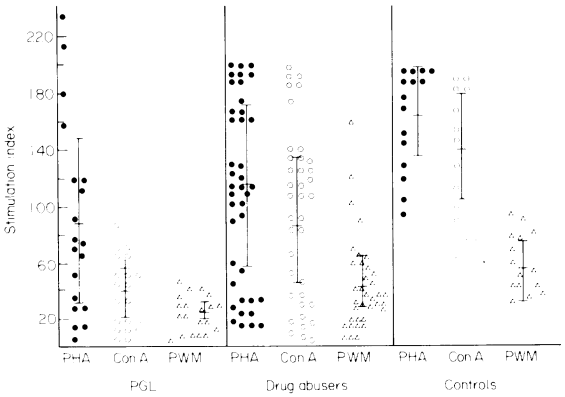

Fig. 1. Response to mitogenic lectins PHA, Con A and PWM (Stimulation index) in PGL and drug abusers compared to healthy controls. Mean values ± s.d. are also shown.

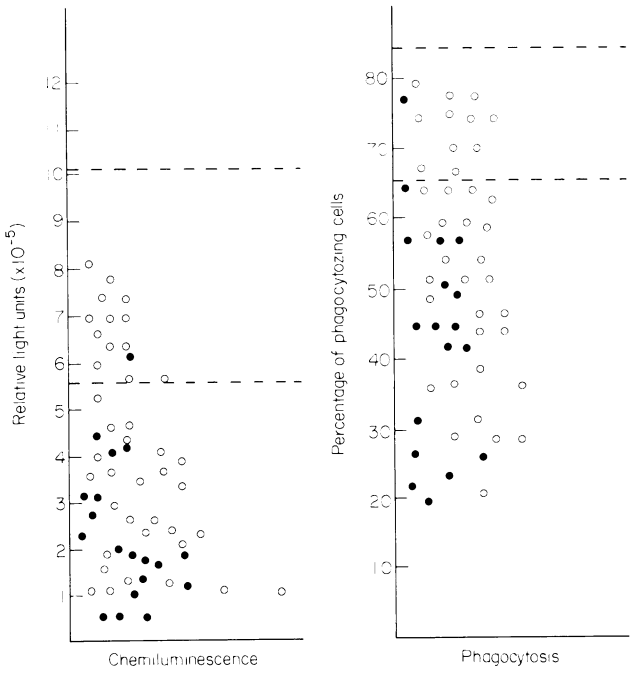

Fig. 2. Chemiluminescent response (Relative light units) and phagocytosis (percentage of phagocytozing cells) of PGL patients (●) and i.v. drug abusers (○). Normal values (mean ± s.d.) are defined by dashed lines.

Table 2. IgG antibody titres against Herpes viruses in drug abusers with or without PGL

	Number of patients with antibodies to:						
	Herpes simplex		Cytomegalovirus		Epstein–Barr virus		
Titre	Drug abusers	PGL	Drug abusers	PGL	Titre	Drug abusers	PGL
Negative	6	2	30	4	Negative	1	1
1/5	8	4	8	6	1/160	14	3
1/10	3	8	6	5	1/320	8	1
1/20	23	3	3	3	1/640	20	4
1/40	10	3	3	2	1/1280	7	8
					1/2560	0	3

patients and healthy controls. The results are expressed as a stimulation index (SI). This index was reduced with all mitogens in PGL patients while variable responses were found in the drug abusers. Natural killer activity was reduced in PGL patients (14% at 12:1 ratio & 34% at 50:1 ratio *v* 36% & 72% respectively in controls) and within the normal range in drug abusers (32% & 67% respectively). Figure 2 shows that the percentage of phagocytizing cells was reduced in the majority of the drug abusers, particularly in the PGL patients. Similar results were obtained with the chemiluminescent response. The serological findings are summarized in Table 2. High titres of anti-Epstein–Barr virus were found both in PGL patients and drug abusers, while IgM were absent. Complement fixing anticytomegalovirus antibodies were negative in the majority of the patients.

The presence of antibodies to HTLV-III was demonstrated by the ELISA technique in 18/20 PGL patients and in 23/50 drug abusers without PGL. These data confirm the previous observation of a high incidence of anti-HTLV-III antibodies in italian heroin addicts (Aiuti, 1984; Rossi *et al.*, 1984). Circulating immune complexes were present in 50% of PGL patients and 30% of asymptomatic drug abusers.

DISCUSSION

We have described the immunological profile of intravenous drug abusers with or without PGL in north east Italy. None of our patients had illnesses known to alter their immunological reactivity, such as infectious mononucleosis or active hepatitis. Ninety per cent of the subjects with PGL had detectable levels of anti HTLV-III antibodies; furthermore these patients had mild lymphopaenia with reversed T4/T8 ratios, Leu-11b positive cells were almost absent and the natural killer activity against the K 562 cell line was significantly reduced at all the effector/target ratios used. The normal values of the Leu-7 positive population complement the previous observations of a functional heterogeneity of this subset (Tilden *et al.*, 1983; Lanier *et al.*, 1984).

The proliferative response to mitogens was generally depressed, although in the drug abusers without PGL there was much individual variation. Finally, neutrophil chemiluminescence and phagocytosis were also depressed. No correlations were found between high titres of anti-EBV, cytomegalovirus or herpes simplex antibodies and reversed T4/T8 ratios or impaired mitogen responsiveness.

The presence of immunological abnormalities in i.v. drug abusers hospitalized for infectious endocarditis or other infections has already been reported (Layon *et al.*, 1984). Among our non-hospitalized drug abusers the immunological abnormalities appear to be similar to those observed in other high risk groups for AIDS (Gottlieb *et al.*, 1981; Masur *et al.*, 1981; Miller *et al.*, 1984; Pinching, 1984). The presence of circulating immune complexes and the depressed neutrophil function were unexpected; patients receiving only maintenance methadone therapy do not have

immune complexes and have only slightly reduced neutrophil function. Furthermore healthy male homosexuals from our region do not show abnormal neutrophil activity, despite the presence of a detectable amount of immune complexes (data not shown). We do not yet know if these abnormalities are correlated to the presence of high susceptibility to microbial infections.

The recognition of PGL in drug abusers with antibodies to HTLV-III is important because such patients may develop AIDS (Center for Disease Control Task Force, 1982; Cavaille Coll *et al.*, 1984). Long term follow up studies are therefore in progress.

Although cell-mediated immunity is more reduced in drug abusers with PGL as compared to those without lymphadenopathy, these differences are not significant (Cavaille Coll *et al.*, 1984). Furthermore there is marked individual variation of cell-mediated immunity responses in the latter, suggesting that only certain patients could be at risk for developing PGL.

In conclusion, the recognition of different immunological patterns in i.v. drug abusers could have important applications in delineating risk factors for developing PGL or AIDS. Our data suggest that AIDS will become common among drug abusers in north east Italy.

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