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Lymphocytotoxic antibodies in patients with inflammatory bowel disease and their spouses—evidence for a transmissible agent

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SUMMARY

Serum lymphocytotoxic antibodies (LCA) were detected in twenty-seven out of fifty-three (51%) patients with inflammatory bowel disease (IBD) and in twenty-three out of their fifty-three (43%) unaffected spouses. The prevalence of LCA in both groups was significantly increased ($P < 0{\cdot}001$) compared to that in age- and sex-matched controls (11%) or in control spouses (6%). Concordant expression of LCA occurred in sixteen out of the fifty-three (30%) patient–spouse pairs compared to only one out of the fifty-three (2%) control–spouse pairs ($P < 0{\cdot}001$). In contrast to the LCA results, heterophile antibody titres were similarly distributed in all four study groups.

It is suggested that LCA may represent markers of infectious agents in IBD and that their occurrence in unaffected close contacts of patients may indicate transmission of such agents to these subjects.

INTRODUCTION

Serum lymphocytotoxic antibodies (LCA) occur in a wide variety of clinical disorders including, notably, acute viral infections, chronic rheumatic disorders (particularly systemic lupus erythematosus) and malignant disease (Messner, 1975). Our previous studies have documented a high prevalence of LCA in patients with inflammatory bowel disease, including both Crohn's disease and chronic ulcerative colitis (Korsmeyer *et al.*, 1974; Strickland *et al.*, 1975). We have characterized these antibodies as cold-reactive non-HLA-dependent, reactive against both T and B cells and of the IgG or IgM class (Korsmeyer *et al.*, 1974; Strickland *et al.*, 1975; Henderson *et al.*, 1976). No relationship between LCA and clinical disease indices such as activity, duration, extent or treatment mode were observed in an earlier study (Strickland *et al.*, 1975). Previous family studies of LCA in inflammatory bowel disease revealed a significant occurrence in serum from unaffected relatives (Korsmeyer *et al.*, 1975). Household contacts of patients showed a higher LCA prevalence than non-household contacts, and the prevalence in a small group of spouses studied approached that seen in patients with inflammatory bowel disease (Korsmeyer *et al.*, 1975). The present study was undertaken to examine this latter observation in more detail.

MATERIALS AND METHODS

Fifty-three patients with well-established inflammatory bowel disease were studied. Thirty-three had Crohn's disease and twenty had chronic ulcerative colitis. The patients were from three different geographic locations in the U.S.A., namely, Albuquerque, New Mexico (twenty-six), Chicago, Illinois (nineteen), and Minneapolis, Minnesota (eight). The spouses of these fifty-three patients were also studied. None had a history of, or clinical evidence for, inflammatory bowel disease.

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Each patient was age- and sex-matched with a control subject selected from the corresponding geographic region. Spouses of these control subjects were also studied. None of the patient spouses, controls or control spouses were suffering from chronic illnesses known to be associated with the occurrence of LCA.

Serum was obtained from all subjects in the four study groups, aliquoted and stored at −20°C prior to testing. The presence of lymphocytotoxic antibody (LCA) was determined by the microcytotoxicity assay of Terasaki & McClelland (1964). With this technique, 1 μl of serum and 1 μl of whole lymphocytes (obtained by Ficoll–Hypaque gradient separation of peripheral venous blood) at 10^6 cells/ml are incubated in wells of a microcytotoxicity plate at 15°C for 15 min; 5 μl of rabbit complement is then added and following a further incubation at 15°C for 3 hr, 3 μl of eosin Y dye and 8 μl of 40% formalin are added sequentially to each well. The percentage of cells killed is determined by dye exclusion using inverted phase-contrast microscopy. Each serum was tested against a standard panel of lymphocytes from twenty normal adult subjects. An individual test with a given serum was considered positive if 20% or more of the target lymphocytes from a single donor were killed. Serum from any given subject was considered to be positive for LCA if positive cytotoxicity was observed in 50% or more of the standard twenty-donor lymphocyte panel (Strickland *et al.*, 1975; Korsmeyer *et al.*, 1975).

All sera were also tested for heterophile antibody using standard laboratory methodology (Davidsohn & Lee, 1964). This IgM antibody was chosen as an additional marker of humoral immunological reactivity.

Statistics. The data were analysed using McNemar's χ^2 test for matched data (Siegel, 1956) and the paired Student's *t*-test.

RESULTS

The results of LCA testing in the fifty-three patients and age- and sex-matched control subjects are shown in Fig. 1. Serum from twenty-seven out of the fifty-three patients (51%) showed LCA (i.e., positive

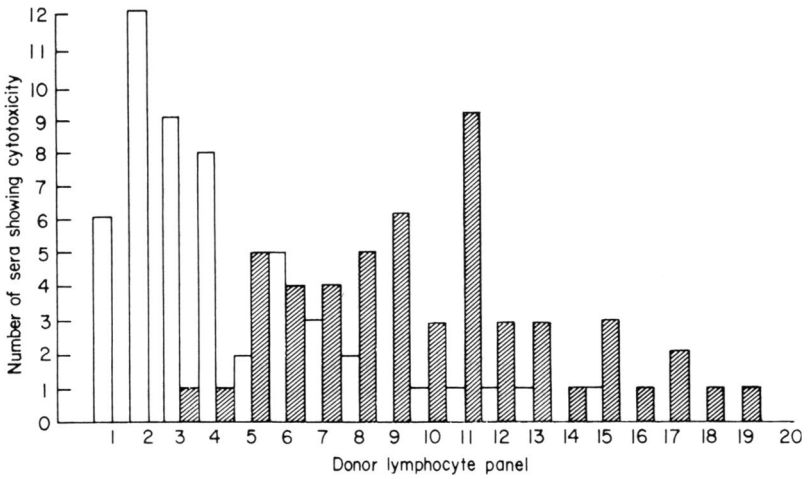

FIG. 1. Distributions of serum lymphocytotoxicity in fifty-three patients with inflammatory bowel disease and in fifty-three age- and sex-matched control subjects. Bars represent the number of sera in each group showing cytotoxicity against from none up to twenty of the donor lymphocyte panel. (□) Controls, total = 53; (▨) IBD patients, total = 53.

cytotoxicity against ten or more of the twenty-donor panel). This included thirteen out of the thirty-three patients with Crohn's disease (40%) and fourteen out of the twenty with chronic ulcerative colitis (70%). Six out of the fifty-three control subjects (11%) showed LCA. The difference in prevalence of LCA between patients and controls was significant ($\chi^2 = 17{\cdot}39$; $P<0{\cdot}001$). Fig. 1 also shows that the overall distribution of lymphocytotoxicity in the patient sample differs substantially from that in the control sample. This observed difference is statistically significant ($t = 8{\cdot}85$; $P<0{\cdot}001$).

Fig. 2 shows corresponding results of LCA testing in the fifty-three unaffected spouses of patients with inflammatory bowel disease compared to those in the control spouses. Twenty-three out of the fifty-three patient spouses (43%) showed LCA. This included seventeen out of the thirty-three Crohn's disease spouses (51%) and six out of twenty ulcerative colitis spouses (30%). Only three out of the fifty-three control spouses (6%) showed LCA. This difference in LCA prevalence between patient and control

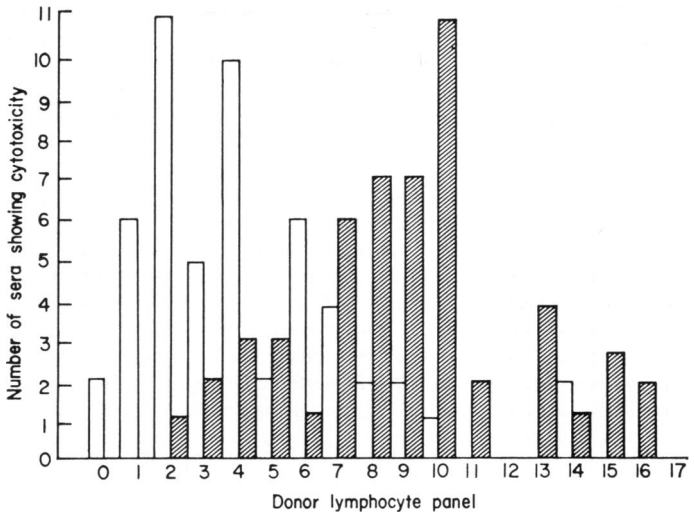

FIG. 2. Distributions of serum lymphocytotoxicity in the fifty-three unaffected spouses of patients shown in Fig. 1 and in fifty-three spouses of the control subjects. Bars represent the number of sera in each group showing cytotoxicity against from none up to twenty of the donor lymphocyte panel. (□) Control spouses, total = 53; (▨) IBD spouses, total = 53.

TABLE 1. Frequency and concordance of lymphocytotoxic antibodies (LCA) in patient–spouse pairs compared to control–spouse pairs

	Number tested	Number of pairs showing LCA	Number of pairs concordant for LCA
Patient–spouse pairs	53	34 (64%) ($P<0.001$)	16 (30%) ($P<0.001$)
Control–spouse pairs	53	8 (15%)	1 (2%)

TABLE 2. Heterophile antibody titres in patients with inflammatory bowel disease (IBD), control subjects, IBD spouses and control spouses

Heterophile titre	IBD patients*	Controls*	IBD spouses*	Control spouses*
< 1/7	24	35	24	32
1/7	17	7	7	13
1/14	7	9	17	6
1/28	3	1	3	0
1/56	2	0	1	1
1/112	0	1	1	1

* Total = 53.

spouses was highly significant ($\chi^2 = 15.04$; $P<0.001$). Fig. 2 again shows that the overall distribution of lymphocytotoxicity amongst patient spouses differs from that in control spouses. This difference is statistically significant ($t = 7.74$; $P<0.001$).

The pattern of occurrence of LCA in the patient–spouse pairs and control–spouse pairs is shown in Table 1. LCA was detected in one or both of thirty-four out of the fifty-three (64%) patient–spouse pairs compared to eight out of the fifty-three (15%) control–spouse pairs ($\chi^2 = 20.83$; $P<0.001$).

Concordant occurrence of LCA was observed in sixteen out of the fifty-three (30%) patient–spouse pairs and in only one out of the fifty-three (2%) control–spouse pairs ($\chi^2 = 11{\cdot}53$; $P<0{\cdot}001$). Amongst the eighteen patient–spouse pairs showing discordant occurrence of LCA, the patient alone was positive in eleven instances and the spouse alone was positive in seven instances.

Table 2 shows the heterophile antibody titres observed in the four study groups. In contrast to the results of LCA testing, no significant differences in heterophile antibody titres were found between patients, their spouses and control subjects or their spouses.

DISCUSSION

The present study confirms our earlier findings of a high prevalence of LCA in the serum of patients with inflammatory bowel disease (Strickland *et al.*, 1975). In addition, the present controlled observations in a large number of unaffected spouses of patients with Crohn's disease or ulcerative colitis substantiate our previous findings of a high frequency of LCA occurring amongst close personal contacts of patients with these disorders (Korsmeyer *et al.*, 1975).

The origin of cold-reactive lymphocytotoxic antibodies which occur in a wide range of clinical disorders is uncertain. Two mechanisms have been proposed (Messner, 1975): (a) LCA arise in response to extreme immunological stimulation and may function as immunoregulatory antibodies; and (b) LCA arise in response to, and are markers of, the presence of infectious agents, either through the sharing of antigenic properties of the agent(s) with surface membrane determinants on the lymphocyte surface, or by the agent interfering with the normal state of tolerance to such determinants. Clearly, mechanism (a) could theoretically account for the presence of LCA in patients with inflammatory bowel disease. However, the presence of LCA in their spouses, who show no evidence of an inflammatory disorder, argues against this proposed mechanism in the present setting. In the present study, the lack of any difference in heterophile antibody titres amongst patients, their spouses and controls stands in sharp contrast to the observations with respect to LCA and provides further evidence against mechanism (a).

Recent studies strongly indicate the presence of RNA viruses in the tissues of patients with either Crohn's disease or ulcerative colitis (Aronson *et al.*, 1975; Gitnick, Arthur & Shibata, 1976; Gitnick & Rosen, 1976). In addition, lymphocytotoxic antibodies are frequently observed transiently in proven acute viral infections (Mottironi & Terasaki, 1970; DeHoratius, Henderson & Strickland, 1976). Preliminary studies also appear to indicate a strong concordance between serum LCA and anti-RNA activity in inflammatory bowel disease families (Korsmeyer *et al.*, 1976), and most recently in patient–spouse pairs (DeHoratius *et al.*, 1977). These observations have led us to consider the possibility that LCA in inflammatory bowel disease arise by mechanism (b) and thus represent a marker of the presence of viruses in these disorders.

Our earlier study of LCA in relatives of patients with inflammatory bowel disease indicated a predominantly horizontal expression of these antibodies in such families (Korsmeyer *et al.*, 1975). The present confirmation of a striking prevalence of LCA in spouses of patients with inflammatory bowel disease, together with frequent concordance of LCA occurrence between individual patients and their spouses, are consistent with the possibility that these antibodies arise in response to infectious agents present in the inflamed bowel, and that these agents are transmissible to close contacts of such patients.

The present findings do not resolve the question of whether viruses in the tissues of patients with inflammatory bowel disease are involved in the pathogenesis of these disorders. If such agents are of aetiological significance, one would need to invoke an additional susceptibility factor to account for the restricted occurrence of inflammatory bowel disease and the virtual absence of these disorders in spouses of affected patients. Alternatively, the chronically inflamed and ulcerated bowel could provide an excellent source for penetration of common viral agents. Secondary viral propagation in inflammatory bowel disease tissues, with subsequent transmission to close contacts, could equally well account for the pattern of occurrence of LCA that we have observed in this and an earlier study (Korsmeyer *et al.*, 1975) in patients with inflammatory bowel disease, their spouses and other unaffected family members.

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