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Enumeration of lymphocyte populations defined by surface markers in the whole blood of patients with Crohn's disease

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SUMMARY The proportions and absolute numbers of different lymphocyte populations were determined using alkaline phosphatase-labelled reagents in the whole peripheral blood of 22 patients with Crohn's disease. Monoclonal mouse anti-T cell antibody (OKT3) was used to identify T cells, polyvalent $F(ab')_2$ anti-human immunoglobulin for B cells, C3b for C3b-receptor bearing cells, and soluble IgG antibody-antigen complexes for Fc(γ)-receptor bearing cells. Endogenous myeloperoxidase served to distinguish monocytes. Application of this methodology to whole blood avoids the inevitable loss of cells which accompanies separation of mononuclear cells from blood and therefore permits precise enumeration of lymphocyte populations in the circulation. No significant difference from healthy adult controls was observed in any of the lymphocyte subsets tested.

The cause and pathogenesis of Crohn's disease are not known but the inflammatory infiltrate in lesions of the disease, consisting of T lymphocytes, B lymphocytes, plasma cells, macrophages, and granulomata,[1,2] suggests that immunological mechanisms may be involved. There is, however, controversy in the literature regarding the *in vivo* immunocompetence of patients with Crohn's disease and the proportions, numbers and functional activity of their peripheral blood lymphocyte populations tested *in vitro*.[1,3–25] All the *in vitro* studies have been performed on preparations of mononuclear cells isolated from peripheral blood. There is no procedure for such isolation that recovers all the mononuclear cells and it is well established that with yields of less than 70% there is appreciable distortion of the relative proportions of T and B lymphocytes,[26] and possibly of subsets within these populations. These distortions make it impossible to extrapolate back to the true proportions and thence to the absolute numbers of different lymphocyte populations in the circulation *in vivo*, which is the clinically relevant information. Some authors, realising the significance of presentation of the absolute values, have calculated them but, in the absence of results detailing lymphocyte yields over 70%, these calculations are not valid. Such high yields are particularly difficult to achieve from the blood of individuals with active inflammatory disease.

Another important problem in enumerating circulating lymphocyte populations is the distinction between lymphocytes and monocytes. The latter share some surface phenotypic properties with lymphocytes, they are very difficult to eliminate completely from lymphocyte preparations, and procedures for their elimination generally cause differential loss of T and B cells.[26]

In order to overcome these problems we have developed methods for identification of lymphocyte surface markers in whole peripheral blood under conditions in which there is no loss of cells.[26–28] Positive cytochemical identification of monocytes by staining for myeloperoxidase enables them to be excluded from the counts and lymphocyte surface markers are identified using alkaline phosphatase-labelled reagents.[29,30] The use of immunoenzyme staining also provides a greatly enhanced sensitivity for detection of some markers, particularly surface immunoglobulin.[31] This has recently enabled us to confirm[31] the observations of Haegert and Coombs[32]

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that the so-called non-B, non-T or 'null' cells in normal peripheral blood are in fact B cells (B-minor cells) which express much lower levels of intrinsic immunoglobulin on their surface than do the classical B cells (B-major cells).

We have now applied this methodology to the precise quantification of peripheral blood lymphocyte populations in patients with Crohn's disease and report here that the proportions and the absolute numbers of T cells, B-major and B-minor cells, Fc(γ) and C3b-receptor cells were not significantly different from normal.

Methods

PATIENTS

Twenty-two patients were studied in whom the diagnosis of Crohn's disease was established on the basis of standard clinical, radiological, and histopathological features, the latter according to the criteria of Lockhart-Mummery and Morson.[1 33 34] There were 15 male and seven female patients with a mean age of 41 years (range 25–78 years). The site of disease at the time of study was the ileum in eight, the colon in six, and ileocolonic in eight. Nine individuals had had bowel resections in the past. At the time when blood was taken for lymphocyte studies, samples were also obtained for measurement of serum C-reactive protein, and the patients were assessed for calculation of the Crohn's disease activity index.[35] The C-reactive protein levels were measured by electroimmunoassay as described previously.[36 37] Healthy adult hospital staff served as controls and one was included in each run of Crohn's disease patients.

Venous blood anticoagulated with EDTA was processed for the alkaline phosphatase-whole blood method precisely as described before.[29–31] T cells were detected using a monoclonal mouse antibody (OKT3, Ortho Pharmaceutical Ltd, High Wycombe, Bucks., England) reactive with all human peripheral blood T cells.[38] B-major and B-minor cells and lymphocytes bearing receptors for Fc(γ) and for C3b were detected using the same reagents and methods as before.[30 31] All the anti-immunoglobulin reagents and the anti-C3 antibody were pepsin $F(ab')_2$ fragments of IgG. The proportion and absolute number of each lymphocyte population defined by a particular surface marker were calculated and are shown in the results.

Results

The percentages and absolute numbers in whole blood of the different lymphocyte populations defined by the various surface markers tested are shown in Tables 1 and 2. There was no significant difference between the whole group of Crohn's disease patients and the control group for any of the markers. The results in patients with active disease were also compared with those in patients with quiescent disease. Two separate analyses were performed, one with Crohn's disease activity index values greater than 149 as the criterion of activity, the other on the basis of serum C-reactive protein concentrations greater than 29 mg/l (Table 3). In neither case were there any significant differences. There was a wider range of total lymphocyte counts in the patient group and therefore more variation in the absolute numbers of the various lymphocyte subsets but there were no significant differences from the controls.

Discussion

The patients studied here were well characterised in terms of the extent and activity of their disease, and included a spectrum of disease activity from quiescent to very active. Using a precise and sensitive method for detection and enumeration of different lymphocyte populations, which avoids the pitfalls of the techniques used in earlier studies, we

Table 1 *Lymphocyte populations identified by surface markers (percentage of total lymphocytes)*

	Crohn's disease				*Normal controls*			
Surface marker	*Mean*	*SD*	*Range*	*No.*	*Mean*	*SD*	*Range*	*No.*
OKT3 positive (T cells)	78·2	7·2	67·0–91·0	18	79·5	6·8	67·3–88·0	8
Surface membrane immunoglobulin detected directly (B-major cells)	15·5	8·1	5·0–31·6	22	14·3	6·1	7·0–27·8	10
Surface membrane immunoglobulin detected with enhanced sensitivity (B-major + B-minor cells)	22·3	8·8	13·0–34·0	7	24·0	2·8	20·9–27·5	4
Sum of T cells + all B cells	97·3	8·1	81·0–104·8	7	99·3	5·9	93·9–107·8	4
C3b-receptor	6·2	3·1	2·0–13·7	22	7·9	3·0	4·0–11·8	10
Fc(γ)-receptor	6·0	4·4	1·0–17·7	22	8·0	2·2	5·0–10·9	10

Table 2 *Lymphocyte populations identified by surface markers (absolute numbers ($\times 10^6$/l))*

	Crohn's disease				*Normal controls*			
Surface marker	*Mean*	*SD*	*Range*	*No.*	*Mean*	*SD*	*Range*	*No.*
Total lymphocyte count	3248	1521	1209–7500	22	2297	610	1400–3383	10
OKT3 positive (T cells)	2392	1078	968–4608	18	1776	452	1167–2353	8
Surface membrane immunoglobulin detected directly (B-major cells)	482	281	89–1191	22	352	226	197–939	10
Surface membrane immunoglobulin detected with enhanced sensitivity (B-major + B-minor cells)	790	335	231–1280	7	553	208	349–818	4
C3b-receptor	189	111	35–449	22	180	68	56–282	10
Fc(γ)-receptor	172	122	23–526	22	180	64	80–285	10

Table 3 *Classification of patients with Crohn's disease according to indices of disease activity*

	Crohn's disease activity index (score)				*C-reactive protein (mg/l)*			
	Criterion	*Median*	*Range*	*No.*	*Criterion*	*Median*	*Range*	*No.*
Active	≥150	188	150–342	7	≥30	43	36–112	8
Quiescent	<150	51	25–140	15	<30	7	0–27	14

were unable to find any difference from normal in the proportions or absolute numbers of T lymphocytes, B-major or B-minor cells, Fc(γ) or C3b-receptor bearing lymphocytes.

These results do not exclude the possibility that individuals with Crohn's disease may have abnormalities among their circulating lymphocytes, but they do show that any such abnormality must be subtle and does not disturb the distribution of the main lymphocyte phenotypes.

It has been suggested that there may be an imbalance of helper and suppressor T cells in this disease[22 25] but the tests used to arrive at this conclusion have been performed on separated mononuclear cell preparations. Furthermore, there is now evidence that some of the so-called Tγ cells, which were characterised as the T suppressor population, are actually monocytes.[39 40] If abnormalities of T cell subsets do exist, a more accurate and reliable way to demonstrate them may be to use the monoclonal antibodies, such as OKT4 and OKT8, which are now available and which specifically recognise helper and suppressor T cells respectively. A whole blood method including positive cytochemical exclusion of monocytes or the application of cytofluorometry to whole blood is also desirable. It is, however, worth noting that in disease states, unlike the normal situation, there may be a significant proportion of T cells which bind both OKT4 and OKT8.[41]

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