

The importance of peripheral immune cells in inflammatory bowel disease

Periferik kan immün hücrelerinin inflamatuvar barsak hastalığındaki önemi

Canan ALKIM, Mustafa BALCI, Hüseyin ALKIM, Ülkü DAĞLI, Erkan PARLAK, Ahmet TEZEL, Aysel ÜLKER

Department of Gastroenterology, Ministry of Health, Yüksek İhtisas Hospital, Ankara

Background/aims: Inappropriate down regulation of an activated immune system is considered as the main pathogenetic mechanism in inflammatory bowel disease. Migration of circulating cells to a diseased intestine is considered as an important factor in the pathogenesis of inflammatory bowel disease. We aimed to evaluate some features of circulating immune cells in inflammatory bowel disease. **Methods:** Twenty-two control, 29 Crohn's disease and 17 ulcerative colitis patients were studied. CD2, CD3, CD4, CD8, CD11b, CD11c, CD25, CD45RA, CD45RO, CD54 and HLA DR on the surface of peripheral blood lymphocytes and CD11b, CD11c, CD45RA and CD45RO on the phagocytes were researched with two-color immunofluorescence flow cytometry. **Results:** The percentages of CD2+ and CD4+ lymphocytes were found significantly reduced in ulcerative colitis. CD3+ and CD8+ lymphocytes in inflammatory bowel disease were higher than in controls. CD45RA+ lymphocytes were found significantly decreased in ulcerative colitis and active Crohn's disease. CD45RO+ lymphocytes and CD45RO+, CD11b+ and CD11c+ phagocytes were significantly increased in Crohn's disease. **Conclusions:** We demonstrated that there were significant differences between ulcerative colitis and Crohn's disease in the expression of some important surface markers on the peripheral blood immune cells. It seems that circulating CD11b-CD11c and CD45RA-CD45RO expressing phagocytes are important in inflammatory bowel disease and may be useful in distinguishing Crohn's disease from ulcerative colitis. These findings may give us some clues about the immunopathogenesis of inflammatory bowel disease.

Key words: Inflammatory bowel disease, Crohn's disease, ulcerative colitis, peripheral blood, lymphocytes, phagocytes, CD subtypes

INTRODUCTION

In inflammatory bowel disease (IBD), possibly through inappropriate down regulation of an activated immune system, the gut is infiltrated by lymphocytes, granulocytes and monocytes that migrate from blood (1-5). Peripheral blood immu-

Amaç: Aktive olmuş immün sistemin uygunsuz down-regülasyonu, inflamatuvar barsak hastalığında ana patogenetik mekanizma olarak kabul edilmektedir. Dolaşımdaki immün hücrelerin hastalıklı barsağa göçü inflamatuvar barsak hastalığının patogeneziinde önemlidir. İnflamatuvar barsak hastalığında, dolaşımdaki immün hücrelerin bazı özelliklerini araştırmayı amaçladık. **Yöntem:** Yirmi iki kontrol, yirmi dokuz Crohn ve on yedi ülseratif kolit hastası incelendi. Periferik kan lenfositleri üzerinde CD2, CD3, CD4, CD8, CD11b, CD11c, CD25, CD45RA, CD45RO, CD54 ve HLA DR ekspresyonu, periferik kan fagositleri üzerinde ise CD11b, CD11c, CD45RA ve CD45RO ekspresyonu iki renkli immünoflüoresans flow sitometri ile çalışıldı. **Bulgular:** CD2+ ve CD4+ lenfositlerin oranı, ülseratif kolitte önemli miktarda azalmış bulundu. CD3+ ve CD8+ lenfositler inflamatuvar barsak hastalığında kontrollerden daha fazlaydı. CD45RA+ lenfositler, ülseratif kolit ve aktif Crohn hastalığında, önemli miktarda azalmış bulundu. CD45RO+ lenfositler ile CD11b+ ve CD11c+ fagositler Crohn hastalığında önemli oranda artmış bulundu. **Sonuç:** Periferik kan immün hücreleri üzerinde bulunan birtakım önemli yüzey markırlarının ekspresyonunda ülseratif kolit ile Crohn hastalığı arasında önemli farklılıklar olduğunu gösterdik. Bulgularımız, dolaşımdaki CD11b-CD11c ile CD45RA-CD45RO ekspresyon eden fagositlerin inflamatuvar barsak hastalığında önemli olabileceği ve Crohn hastalığını ülseratif kolitten ayırt etmede yardımcı olabileceğini düşündürmektedir. Bu bulgular, inflamatuvar barsak hastalığının immünopatogenezi hakkında bize bazı ip uçları verebilir.

Anahtar kelimeler: İnflamatuvar barsak hastalığı, Crohn hastalığı, ülseratif kolit, periferik kan, lenfosit, fagosit, CD alt tipleri

ne cells may be considered as a part of the intestinal immune system. Perlmann and Broberger's (6) in vitro study showed that the peripheral white blood cells of ulcerative colitis (UC) patients have a cytotoxic effect on human fetal colon cells. Pallo-

Address for correspondence: Canan ALKIM

Department of Gastroenterology, Ministry of Health, Yüksek İhtisas Hospital, Ankara, Turkey

Phone: +90 212 233 94 46 • Fax: +90 212 252 84 49

E-mail: alkimca@hotmail.com

Manuscript received: 27.10.2006 Accepted: 03.05.2007

ne et al. (7) studied early activation markers on the peripheral blood and intestinal lymphocytes of Crohn's disease (CD) patients and found increased and similar expression of these markers. Also, labelled peripheral blood phagocytes (granulocytes and monocytes) were shown to accumulate rapidly in the diseased gut in IBD patients (8). This movement was correlated with the disease activity. All the peripheral immune cells were found to migrate and to recirculate from intestine to blood and/or from blood to intestine. Migration of circulating cells to diseased intestine is considered as an important factor in the pathogenesis of IBD (1-5,9).

When resting lymphocytes recognize foreign antigen by means of specific receptors on their cell surfaces, they become activated and proliferate or differentiate into effector lymphocytes that can mediate a spectrum of immune effector functions. CD2 (lymphocyte function associated molecule-2, LFA-2) is an adhesion molecule from the immunoglobulin supergene family. The immunoglobulin supergene family plays an important role in recognition and adhesion of cells. It appears to function in triggering T cell activation after antigen presentation. On the T lymphocyte surface, T cell receptor (TCR) is associated with CD3 complex. The CD3 complex is a constellation of at least five transmembrane polypeptides that is required for the transport of TCR to the cell surface and that functions to transmit signals from TCR to the interior of the cell. CD3 complex is present on all T cells and is used as a marker that identifies a lymphocyte as a member of the T-cell lineage. CD4 and CD8 are co-receptor molecules on T cells that function in close association with TCR. CD4+ T cells recognize antigen bound HLA class II molecules, named as helper-inducer T lymphocytes. CD8+ T cells recognize antigen bound HLA class I molecules, named as cytotoxic-suppressor T cells (10). It has been demonstrated that β 2 integrins play a central role in neutrophil-epithelial adhesive interactions. β 2 integrins are heterodimeric integral membrane glycoproteins expressed only on leukocytes and consist of a common CD18 β chain, which associates with one of four α chains: CD11a, CD11b, CD11c and CD11d (11). CD25 is an α chain of interleukin (IL)-2 receptor, which is expressed on activated T cells, B cells and phagocytes. CD45 R molecule exists in several isoforms on T cells and exhibits phosphotyrosine phosphatase activity. CD45 RA isoform is a marker of a naive or virgin T cell, whereas expression of the CD45

RO isoform is thought to mark a memory or activated T cell. CD54 (ICAM-1) is an adhesive molecule from the immunoglobulin supergene family presented on endothelium and monocytes. HLA class I and II glycoproteins bind peptides and it is this HLA-peptide complex that is recognized by the TCR for antigen on T cells. Class II HLA glycoproteins are presented on the surfaces of the immune system cells. HLA DR is one of HLA class II molecules (10).

The characteristics of circulating cells, for example, the expression of various kinds of adhesion molecules and the states of their activation and maturation, may be important in the pathogenesis of IBD and in the differentiation of CD from UC.

MATERIALS AND METHODS

Twenty-nine patients with CD (12 F, 17 M, mean age 35 ± 10 years), 17 patients with UC (6 F, 11 M, mean age 34 ± 11 years) and 22 healthy controls (11 F, 11 M, mean age 34 ± 11 years) were studied. There was no difference between the groups according to age and sex distribution. CD and UC diagnoses were established based on the clinical symptoms, radiological findings and endoscopic and pathological demonstrations of the inflamed intestinal mucosa.

The disease activity in each patient with CD was analyzed according to Harvey-Bradshaw's index (12). Five or more points were considered to indicate active disease. Thirteen patients had active disease and 16 patients had inactive disease. When the study was performed on the patients with CD, 21 patients were taking only 5-ASA preparations, four were taking the combination of 5-ASA and steroids, and four were not taking any medication.

In UC patients, the disease activity was evaluated according to Rachmilewitz's endoscopic activity index (13). Five or more points were considered to indicate active disease. Seven of the 17 patients had active disease. At the time of study, 12 patients were taking only 5-ASA and five were taking the combination of 5-ASA and steroids.

Whole blood was drawn to K3EDTA vacutainer blood collection tubes. LeucoGATE (CD45/CD14) and isotypic control (G1/G2a) tubes were used for each sample. Eleven parameters (CD2, CD3, CD4, CD8, CD11b, CD11c, CD25, CD54, HLA DR, CD45RA and CD45RO) for lymphocyte population and four parameters (CD11b, CD11c, CD45RA,

and CD45RO) for phagocyte (monocyte plus neutrophil) population were studied. All monoclonal antibodies (Mabs) and isotypic controls were obtained from Becton Dickinson (USA). The study was performed using the directions as provided by the manufacturer. The flow cytometer was calibrated and controlled according to the standard procedures. Acquisition and analysis were performed using a FACScan flow cytometer using Lysis II software (Becton Dickinson, USA). A biparametric gate in the forward scatter-side scatter dot plot was drawn around two cell populations. Fluorescein isothiocyanate (FITC) and phycoerythrin (PE) labelled Mabs were used for fluorescence measurement and 10000 total events counted. With appropriate isotypic control antibodies, fluorescence intensity was defined so that at least 99.5% of gated cells could be counted. All gated cells higher than this threshold were regarded as positive and expressed in the percentage of the cells analyzed.

Student's t test was used for the analysis of the data. A p value of <0.05 was considered to indicate a significant difference between the groups.

RESULTS

Values for each studied marker were presented as mean percent of the cells \pm standard deviation expressing the studied marker. Then, CD, UC and healthy control groups were compared with each other. CD and UC groups were also divided as active or inactive. The active group of each disease was compared with its inactive group and healthy controls. Furthermore, the CD group was divided as short-term and long-term CD groups according to the disease duration (<3 years or >3 years, respectively). The beginning of the CD was accepted as the date of the first symptom. Short- and long-term CD groups were compared with each other and the healthy control group. The results are given in Tables 1 and 2.

There was no significant difference between the groups according to white blood cell number. The percentage of total lymphocytes of long-term CD patients was found significantly lower than of short-term CD patients ($p=0.002$). Phagocytes percentage in long-term CD patients was significantly higher than in the short-term CD group ($p=0.003$).

Table 1. Expression of the different markers on the peripheral blood lymphocytes in inflammatory bowel disease

	CD2 (%)	CD3 (%)	CD4 (%)	CD8 (%)	CD45RA (%)	CD45RO (%)	CD54 (%)
HC	76.1 \pm 12.3	65.9 \pm 9.1	35.5 \pm 6.1	29.1 \pm 7.1	45.8 \pm 9.2	28.7 \pm 8.6	34.7 \pm 12.1
UC	65.3 \pm 16.8 ^b	72.1 \pm 13.1	30.2 \pm 8.8 ^{ab}	37.8 \pm 12.6 ^a	37.6 \pm 11.7 ^a	29.9 \pm 10.4	21.3 \pm 12.7 ^b
Active UC	53 \pm 12.2 ^a	66 \pm 12.1	30.3 \pm 12.6	33.6 \pm 11.5	37.6 \pm 10.6 ^a	35 \pm 12.8	13.2 \pm 13 ^{ac}
Inactive UC	73.9 \pm 16.6	76.4 \pm 12.5 ^a	30.2 \pm 5.8	40.7 \pm 13 ^a	37.6 \pm 11.8 ^a	26.4 \pm 19.8	26.9 \pm 10.5 ^c
CD	78.5 \pm 8.6 ^b	73.8 \pm 8.7 ^a	35.9 \pm 10.3 ^b	37.5 \pm 10.7 ^a	44.3 \pm 10.2	33.7 \pm 13.4	11.9 \pm 8.2 ^{ab}
Active CD	78.9 \pm 8.8	75.1 \pm 8.1 ^a	35.1 \pm 11.5	38.7 \pm 11.2 ^a	38 \pm 7.8 ^{ad}	39.7 \pm 11.9 ^d	16.1 \pm 8.8 ^{ad}
Inactive CD	78.1 \pm 8.6	72.8 \pm 9.3 ^a	36.6 \pm 9.5	36.5 \pm 10.5 ^a	48.7 \pm 9.9 ^d	29 \pm 13.1 ^d	8.3 \pm 7.6 ^{ad}
Short CD	77.9 \pm 9.7	72.9 \pm 7.7 ^a	35.8 \pm 9.1	37 \pm 10.7 ^a	49.3 \pm 9.9 ^e	32.3 \pm 12.8	13.7 \pm 7.5 ^a
Long CD	78.9 \pm 7.7	74.6 \pm 9.7 ^a	36 \pm 11.5	37.9 \pm 10.9 ^a	39.7 \pm 8.4 ^{ae}	35.1 \pm 14.4	10.4 \pm 6.4 ^a

HC: Healthy control, UC: Ulcerative colitis, CD: Crohn's disease, Short: Disease time <3 years, Long: Disease time >3 years, $p<0.05$ a: vs HC, b: UC vs CD, c: Active vs inactive UC, d: Active vs inactive CD, e: Short vs long CD

Table 2. Expression of the different markers on the peripheral blood phagocytes (granulocytes and monocytes) in inflammatory bowel disease

	WBC (n)	Lymph (%)	Phago (%)	CD11b (%)	CD11c (%)	CD45RA (%)	CD45RO (%)
HC	9900 \pm 1363	27.9 \pm 5.2	70.1 \pm 5.4	60.3 \pm 9.8	45 \pm 8.5	2.3 \pm 1.8	20.9 \pm 7.3
UC	8841 \pm 2315	27.4 \pm 9	69.4 \pm 10.7	58.8 \pm 10.7 ^b	49.8 \pm 9.6 ^b	0.9 \pm 0.7	9.1 \pm 3.9 ^{ab}
Active UC	9700 \pm 2754	26.3 \pm 9.3	70.6 \pm 12.8	40 \pm 12.1 ^{ac}	35 \pm 8.6 ^{ac}	0.8 \pm 0.5	9.9 \pm 3.7 ^a
Inactive UC	7560 \pm 1540	28.1 \pm 9.2	68.5 \pm 9.7	70.1 \pm 12.2 ^c	58.7 \pm 9.8 ^c	0.9 \pm 0.8	8.6 \pm 4.1 ^a
CD	8800 \pm 3224	26.1 \pm 10.1	72.1 \pm 10.7	81.2 \pm 12.1 ^{ab}	65 \pm 10.8 ^{ab}	1.2 \pm 0.9	32.6 \pm 7.4 ^{ab}
Active CD	9939 \pm 3780	23.1 \pm 9.5	75.5 \pm 9.8	84.6 \pm 12.2 ^a	71.2 \pm 9.9 ^a	1.6 \pm 0.9 ^d	38 \pm 8.1 ^{ad}
Inactive CD	7875 \pm 2436	28.5 \pm 10.1	69.4 \pm 10.8	78.4 \pm 11.2 ^a	60.1 \pm 10.2 ^a	0.9 \pm 0.6	28.2 \pm 6.8 ^{ad}
Short CD	8131 \pm 2661	32.1 \pm 8.3 ^e	65.8 \pm 8.2 ^e	76.1 \pm 12.5 ^{ae}	53.2 \pm 12.8 ^{ae}	1.7 \pm 0.9	33.6 \pm 7.3 ^a
Long CD	9344 \pm 3610	21.2 \pm 8.8 ^e	77.2 \pm 9.8 ^e	85.3 \pm 11.4 ^{ae}	74.6 \pm 13.9 ^{ae}	0.8 \pm 0.5	31.6 \pm 7.6 ^a

HC: Healthy control, UC: Ulcerative colitis, CD: Crohn's disease, Short: Disease time <3 years, Long: Disease >3 years, $p<0.05$ a: vs HC, b: UC vs CD, c: Active vs inactive UC, d: Active vs inactive CD, e: Short vs long CD

The percentage of CD2+ lymphocytes of UC patients was remarkably lower than in CD and healthy control groups, but only the difference between UC and CD groups was statistically significant ($p=0.02$). When UC patients were compared according to the disease activity, the CD2 percentage of lymphocytes of the inactive group was similar to that of the healthy control group, but the active group had the lowest CD2 positivity and this was significantly lower than in the healthy control and inactive UC groups ($p<0.05$). CD2 positivity of the CD subgroups and the healthy control group was similar.

The percentages of CD3+ lymphocytes were found increased in both of the IBD groups when compared with healthy controls, and the difference between CD and healthy control groups was statistically significant ($p=0.003$). While CD3 positivity of the active UC group was similar to that of the healthy control group, the value of the inactive group was significantly higher. All the CD subgroups had statistically higher values than the healthy control group. There was no difference between the CD subgroups.

The CD4 positivity of the UC group was the lowest, and this was significantly different from the CD and healthy control groups ($p<0.05$). The CD and healthy control groups had similar values. The disease activation did not influence CD4 percentage in the UC and CD groups.

The percentage of CD8+ lymphocytes was found significantly increased in both UC and CD groups compared to the healthy control group. In the CD group, CD8 positivity did not change with disease activity or disease duration. All the CD subgroups had higher values than the healthy control group with statistical significance. On the other hand, in the UC group, CD8 positivity of the inactive group was found increased when compared with the active group, without statistical significance. There was a significant difference between the inactive UC and healthy control groups, but there was no difference between the active group and the healthy control group.

Detailed data about the expressions of HLA DR and CD25 were not given because there was no difference between the groups. In brief, all the groups had 15-17% of HLA DR positivity and 5-9% of CD25 positivity on their lymphocytes.

CD11b and CD11c were studied on the surface of both lymphocytes and phagocytes. CD11b+ or

CD11c+ lymphocytes were found similar in all groups (between 12-16% and 17-21%, respectively). Again, detailed data were not given. On the phagocytes, CD11b positivity of the CD group was statistically higher than of the UC and healthy control groups ($p=0.004$). UC and healthy control group values were similar. Active CD had higher CD11b+ phagocytes than inactive CD, but this difference was not statistically significant. All the CD subgroups had significantly higher values than the healthy controls. The long-term CD group had significantly higher expression of CD11b on their phagocytes when compared to short-term CD. Active UC had the lowest positivity of CD11b on their peripheral phagocytes and this was significantly lower than in the inactive UC and healthy control groups. The distribution of CD11c expression on phagocytes was similar to CD11b distribution. It must be emphasized that CD11b and CD11c expressions in all the CD groups were significantly higher than in the UC and healthy control groups, and the lowest expression was in the active UC group.

Again, CD45RA and CD45RO expressions were studied on both lymphocytes and phagocytes. CD45RA+ lymphocytes were found reduced in the UC group when compared with CD and healthy controls. However, only the difference between the UC and healthy control groups was statistically significant ($p=0.05$). This data did not change with the disease activity in UC. While the percentage of CD45RA+ lymphocytes of total CD was similar to that of healthy controls, the expression of CD45RA on the lymphocytes of active and long-term CD subgroups was found decreased obviously and these values were significantly lower than in healthy controls ($p=0.01$). Also, the differences between active and inactive CD subgroups and short- and long-term CD subgroups were statistically significant. The proportion of CD45RO+ lymphocytes was not different between CD, UC and healthy controls. Within the CD group, CD45RO+ lymphocytes were found increased significantly in active CD when compared with inactive CD and healthy controls. There was no difference between short- and long-term CD.

The expression of CD45RA on phagocytes was very low and we could not find any difference between the groups. Mean CD45RO expression of peripheral phagocytes was below 10% in all the UC groups and these values were significantly lower than in healthy controls and CD. On the contrary,

mean CD45RO expression of phagocytes was above 28% in all the CD groups and these values were significantly higher than in healthy control and UC groups ($p < 0.05$). In addition, active CD had the highest expression of CD45RO on their phagocytes and this was significantly higher than in inactive CD.

Finally, CD54 (ICAM-1) positivity was evaluated on the lymphocytes. CD54 expression was significantly reduced in CD, compared to UC and healthy controls. Although CD54 positivity of UC was lower than in healthy controls, this did not reach statistical significance. However, the active UC group had significantly lower CD54 expression than healthy controls. While the active CD subgroup had significantly higher CD54+ lymphocytes, the active UC subgroup had significantly lower CD54+ lymphocytes, when compared to their respective inactive groups. Furthermore, CD54 expressions of all the CD subgroups were significantly lower than of the healthy control group.

DISCUSSION

In the present study, we found some important clues about IBD through peripheral blood. Firstly, we compared all groups according to total white blood cells and percentages of lymphocytes and phagocytes and we found no difference between groups, other than decreased lymphocytes and increased phagocytes in long-term CD patients. Auer (14) and Selby (15) reported lymphopenia in IBD. Increased phagocytes were reported previously in all the IBD groups as a part of acute phase response, but we found statistically significant elevation only in active or long-term CD groups. This result may suggest an important role of phagocytes in IBD, especially in CD.

Secondly, we compared lymphocyte subtypes. We found that CD3+ lymphocytes (total T cells) were increased in all the IBD groups. CD3 positivity of all the IBD groups was found significantly higher than in healthy controls, except for the active UC group.

In the present study, we found that UC had lower CD4+ helper-inducer lymphocytes than CD and healthy controls. In addition, CD8+ cytotoxic-suppressor T cells were elevated in both UC and CD, unrelated to disease activity. Therefore, the CD4/CD8 ratio was found significantly decreased in the UC group. According to the literature, CD4+ lymphocytes were reduced in both UC and active

CD groups, CD8+ lymphocytes were reduced only in the active CD group, and finally the CD4/CD8 ratio did not change in IBD (15). Our findings were somewhat different. In the present study, CD3+ T lymphocytes and CD8+ cytotoxic-suppressor T lymphocytes were increased and CD4 positivity did not change in the CD group. But, in the UC group, we found that while CD4+ helper-inducer lymphocytes decreased, CD3+ and CD8+ lymphocytes increased. In fact, CD4+ T cells are the predominant cell type that infiltrates the intestine in IBD (16). Thus, the decrease of CD4+ lymphocytes in UC may be related with migration to the intestine.

CD2 plays an important role in the activation and adhesion of the lymphocytes. Upon activation, the expression of CD2 was increasing on the surface of CD4+ and CD8+ lymphocytes. In our study, CD2+ lymphocytes were found decreased in UC, and the active UC subgroup had the lowest value. It is known that CD2 expression mostly takes place on the surface of CD4+ lymphocytes. Thus, the decrease in CD2 expression in the UC group was probably related to the lower CD4 expression. This may be the result of intestinal migration of activated helper T lymphocytes.

CD11b and CD11c are α chains of $\beta 2$ integrins. It was first noted that neutrophils derived from patients with leukocyte adhesive deficiency lacking $\beta 2$ integrins were unable to transmigrate across epithelia (11). Subsequently, experiments using blocking monoclonal antibodies showed that one of these integrins, CD11b/CD18, was required for transmigration (17). Nakamura and colleagues (18) showed that CD11b/CD18+ granulocytes were increased in the intestinal tissue of the patients with IBD. It is confirmed that integrins play an important role in granuloma formation in CD (19). In the present study, CD11b and CD11c expressions of lymphocytes were similar in all the groups. But phagocytes' CD11b and CD11c positivities were significantly increased in CD, and this increase was becoming more evident in active and long-term CD subgroups. Our results are compatible with the literature. We believe that integrins and phagocytes play a very important role in the pathogenesis of CD, and the expression of these molecules may help in differentiating CD from UC.

Recruitment and activation of naive cells in the intestine was reported in CD, as well as recirculation of naive and memory T cells between blood and intestine (20). Meenan (21) and Roman (22) repor-

ted that circulating memory cells were increased in IBD. Yacyshyn et al. (23) found that in CD, CD45RO expression of circulating B lymphocytes was increased, which correlates with increased intestinal permeability, and this expression may be seen before clinical presentation in patients' relatives with increased intestinal permeability. We found that CD45RA+ naive lymphocytes were reduced in UC, active CD and long-term CD groups. Furthermore, CD45RO expression of lymphocytes (memory T cells) was increased only in the active CD group. The decrease of CD45RA expression of lymphocytes may be related with intestinal migration, but this migration seemed to be slower and more continuous in CD than UC. Though we believe that CD45RA and CD45RO expression of lymphocytes is not specific for CD or UC, it does give some important clues in IBD pathogenesis. On the other hand, we found that CD45RO+ phagocytes were significantly higher in CD than the other groups. While the active CD group had the

highest expression of CD45RO, the UC group had the lowest expression. Elevated CD45RO+ phagocytes may be responsible for the distinct clinical presentation of CD.

Soluble ICAM-1 was found increased in IBD and there was elevated ICAM-1+ cells and venules in IBD tissue (24). It has been demonstrated that anti ICAM-1 antibody inhibits monocytes aggregation in vitro in CD (25). In the present study, CD54 expression of circulating lymphocytes was found decreased in CD and active UC.

Peripheral blood is a large cell lake. It may be influenced easily by inflammation in any other area of the body. But, in the peripheral blood, it is important to discover specific markers reflecting intestinal inflammation or differentiating IBD types. The present study showed that CD11b-CD11c and CD45RA-CD45RO, especially on the surface of peripheral phagocytes, were important in IBD patients and they may be candidates for differentiating IBD types.

REFERENCES

- Podolsky DK. Inflammatory bowel disease (first of two parts). *N Engl J Med* 1991; 325: 928-37.
- Sartor RB. Current concepts of the etiology and pathogenesis of ulcerative colitis and Crohn's disease. *Gastroenterol Clin North Am* 1995; 24: 475-507.
- Sartor RB. Cytokines in intestinal inflammation: pathophysiological and clinical considerations. *Gastroenterology* 1994; 106: 533-9.
- Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology* 1998; 115: 182-205.
- Schölmerich J. Inflammatory bowel disease. *Endoscopy* 1999; 31: 66-73.
- Perlmann P, Broberger O. In vitro studies of ulcerative colitis. Cytotoxic action of white blood cells from patients on human fetal colon cells. *J Exp Med* 1963; 117: 717-33.
- Pallone F, Fais S, Squarcia O, et al. Activation of peripheral blood and intestinal lamina propria lymphocytes in Crohn's disease. In vivo state of activation and in vitro response to stimulation as defined by the expression of early activation antigens. *Gut* 1987; 28: 745-53.
- Saverymuttu SH, Camilleri M, Rees H, et al. Indium-111 granulocyte scanning in the assessment of disease extent and disease activity in inflammatory bowel disease. A comparison with colonoscopy, histology and fecal indium-111 granulocyte excretion. *Gastroenterology* 1986; 90: 1121-8.
- MacDermott RP. Alterations in the mucosal immune system in ulcerative colitis and Crohn's disease. *Med Clin North Am* 1994; 78: 1207-31.
- Kagnoff MF. Immunology and inflammation of the gastrointestinal tract. In: Feldman M, Scharschmidt BF, Sleisenger MH, eds. *Sleisenger & Fordtran's gastrointestinal and liver disease*. 6th ed. Philadelphia: W.B. Saunders, 1998; 19-48.
- Madara JL, Sitaraman SV. Intestinal epithelial barrier function: characteristics and responses to neutrophil trans-epithelial migration. In: Sartor RB, Sandborn WJ, eds. *Kirsner's inflammatory bowel diseases*. New York: W.B. Saunders, 2004; 3-17.
- Harvey RF, Bradshaw JM. A simple index of Crohn's disease activity. *Lancet* 1980; 1: 514.
- Rachmilewitz D. Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis. *BMJ* 1989; 298: 82-6.
- Auer IO, Wechsler W, Ziemer E, et al. Immune status in Crohn's disease. 1. Leukocytes and lymphocyte subpopulations in peripheral blood. *Scand J Gastroenterol* 1978; 13: 561-71.
- Selby WS, Jewell DP. T lymphocyte subsets in inflammatory bowel disease: peripheral blood. *Gut* 1983; 24: 99-105.
- Selby WS, Janossy G, Bofill M, Jewell DP. Intestinal lymphocyte subpopulations in inflammatory bowel disease: an analysis by immunohistological and cell isolation techniques. *Gut* 1984; 25: 32-40.
- Parkos CA, Delp C, Arnaout MA, Madara JL. Neutrophil migration across a cultured intestinal epithelium: dependence on a CD11b/18-mediated event and enhanced efficiency in physiological direction. *J Clin Invest* 1991; 88: 1605-12.
- Nakamura S, Ohtani H, Watanabe Y, et al. In situ expression of the cell adhesion molecules in inflammatory bowel disease. Evidence of immunologic activation of vascular endothelial cells. *Lab Invest* 1993; 69: 77-85.
- Mishra L, Mishra BB, Harris M, et al. In vitro cell aggregation and cell adhesion molecules in Crohn's disease. *Gastroenterology* 1993; 104: 772-9.
- Burgio VL, Fais S, Boirivant M, et al. Peripheral monocyte naive T-cell recruitment and activation in Crohn's disease. *Gastroenterology* 1995; 109: 1029-38.
- Meenan J, Spaans J, Grool TA, et al. Altered expression of $\alpha 4\beta 7$, a gut homing integrin, by circulating and mucosal T cells in colonic mucosal inflammation. *Gut* 1997; 40: 241-6.
- Roman LI, Manzano L, De La Hera A, et al. Expanded CD4⁺CD45RO⁺ phenotype and defective proliferative response in T lymphocytes from patients with Crohn's disease. *Gastroenterology* 1996; 110: 1008-19.

23. Yacyshyn BR, Meddings JB. CD45RO expression of circulating CD19⁺ B cells in Crohn's disease correlates with intestinal permeability. *Gastroenterology* 1995; 108: 132-7.
24. Nielsen OH, Brynskov J, Vainer B. Increased mucosal concentrations of soluble intercellular adhesion molecule-1 (sICAM-1), sE-selectin and interleukin-8 in active ulcerative colitis. *Dig Dis Sci* 1996; 41: 1780-5.
25. Liu ZX, Noguchi M, Hiwatashi N, Toyota T. Monocyte aggregation and multinucleated giant-cell formation in vitro in Crohn's disease. The effect of cell adhesion molecules. *Scand J Gastroenterol* 1996; 31: 706-10.