Splenic Lymphoid Change in Abdominal Neoplastic Patients — Analysis of 121 Cases —

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Because the spleen is likely to play a specific role in immunity, we have tried to observe the influence of the abdominal neoplasms on splenic lymphoid tissue as well as the distribution and localization of immunoregulatory cells with a special attention to the marginal zone, using splenectomy specimens in the various kinds of 121 abdominal neoplasm patients. As a control group, twenty-six splenectomy specimens from patients with traumatic rupture were used.

In splenic size and weight, there was a statistically meaningful increase in the patients with abdominal neoplasms. Among those patients, the evolving activated immune reaction(EVA) was 60.2%, the early activated immune reaction(EAA) 39.0%, the mixed evolving activated and granulomatous reaction(MIX) 0.8%, unlike EVA 30.8%, EAA 69.2%, and MIX 0% in the normal control group. The reason for this change may be explained by activated lymphoid tissue in the form of EVA type.

In conclusion, the splenic lymphoid tissue in the various kinds of abdominal neoplasms, mostly malignant, revealed the chronic immune activated state characterized by the increased number of prominent germinal centers and distinct marginal zones, the latter of which revealed the positive reaction for L26, IgM and IgG, and negative for IgD, as well as showing increased natural killer and dendritic reticulum cells identified by Leu7 and S-100 protein respectively. Therefore, we could at least find the significance of the immunologic role of the spleen in the case of abdominal neoplasms, mostly from malignancy.

Key Words : Spleen, Abdominal neoplasms, Lymphoid change.

INTRODUCTION

The human spleen, an organ of unique anatomic

and functional importance, is the largest component of the reticuloendothelial system. And the splenic white pulp (periarteriolar lymphoid sheath, lymphoid follicle, and marginal zone) which constitutes of the highly organized and immunologically active components is affected by many physiologic and pathologic processes(Bishop et al.,1982; Wolf et al.,1989).

The recent development of monoclonal antibodies, directed against lymphoid cell surface and

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cytoplasmic antigens, has been able to characterize various human lymphoid subpopulations. As a consequence of this characterization, it has become possible to analyse the localization and proportion of cell subsets in lymphoid organs (Stein et al., 1980; Poppema et al., 1981; Bhan et al., 1981; Hsu et al., 1983, 1984; Murray et al., 1984). Because the spleen is likely to play a specific role in immunity, we have observed the influence of the abdominal neoplasms on splenic lymphoid tissue as well as the distribution and localization of immunoregulatory cells with a special attention to the marginal zone, the unique compartment localized at the border of the white and red pulp in the spleen, using splenectomy specimens obtained from the patients with various kinds of abdominal neoplasm.

The purposes of this study are firstly to compare the splenic size and weight with those of a normal control group, secondly to classify the types of splenic lymphoid change by examining the splenic lymphoid reaction in the periarteriolar lymphoid sheath, lymphoid follicles and marginal zone, thirdly to analyze the presence and localization of B, T, natural killer cells, macrophages and dendritic cells.

MATERIALS AND METHODS

The splenectomy specimens used in this study were obtained from one hundred and five patients at Inje Univ. Seoul Paik Hospital from January, 1983 to March, 1993 (10 years and 3 months) and sixteen patients at Inje Univ. Sanggye Paik Hospital from September, 1989 to March, 1993 (3 years and 6 months) with various abdominal neoplasm patients undergoing tumor resection and splenectomy

together. Abdominal neoplasms included both malignant and benign tumors of the stomach, pancreas, colon, ovary, kidney, gallbladder and adrenal gland (Table 1), and most of the spleen were accidentally removed. Several kinds of neoplasms, that could affect the splenic structure, such as hepatocellular carcinoma, esophageal carcinoma and primary splenic malignancy, etc. were excluded. As a control group, twenty-six splenectomy specimens from patients with traumatic ruptures were used. Each spleen measured the superior-inferior length (cm) and weight (gms) respectively.

For the histopathologic examination, all the H & E slides except for three cases (poor fixation and improper paraffin blocks) were reviewed. One more H & E section with the corresponding paraffin blocks was done to examine detail and periodic acid-Schiff stain was done when necessary. The splenic lymphoid change was divided into three types i.e. early activated immune reaction, evolving activated immune response and granulomatous reaction, as proposed by Wolf and Neiman (1989).

For the immunohistochemical study, a series of monoclonal antibodies (McAb), such as L26, IgG, IgM and IgD for B lymphocytes, UCHL1 for T lymphocytes, Leu7 for natural killer cells, lysozyme for macrophages and S-100 protein for dendritic cells, was used in an avidin-biotin complex method on formalin fixed and paraffin embedded tissue to analyze the distribution and localization of lymphoreticular cells. Only alpha-naphthyl butyrate esterase stain (ANBE) for monocytes was done by the routine nonspecific esterase method with the frozen tissue section.

Table 1. Total One-hundred-twenty-one Splenectomized Specimens from the Patients with Various Abdominal Neoplasms

Site	Diagnosis (Each No. of cases)	No. of cases (%)
Stomach	Adenocarcinoma 97, NHL* 3, Leiomyoma 1, Leiomyosarcoma 1	102 (84.3)
Colon	Adenocarcinoma 5	5 (4.6)
Pancreas	Mucinous adenoma 3, Islet cell tumor 2, PSEN* 1	6 (5.7)
Ovary	Mucinous adenoma 1, Mature teratoma 1	2 (1.7)
Kidney	Renal cell carcinoma 2, Transitional cell carcinoma 1	3 (2.5)
Gallbladder	Adenocarcinoma 1	1 (0.8)
Adrenal gland	Neuroblastoma 1	1 (0.8)
Unknown	Perisplenic adenocarcinoma 1	1 (0.8)
Total		121 (100.0)

NHL: nonHodgkin's lymphoma, PSEN: papillary and solid epithelial neoplasm

Fig. 1. Age & sex distribution of 121 abdominal & 26 traumatic rupture patients.

RESULT

We observed one hundred and twenty-one surgically resected spleens from patients with abdominal neoplasms as a study group and twenty six splenectomy specimens from patients with traumatic ruptures as a normal control group.

The patients' ages in the study group varied from 1-77 years and the average age was 54 years. In contrast, that of the control group was 4-68 years and the average age was 35 years. Male to female ratio was 1.4 : 1 in the study group and 2.3 : 1 in the control group respectively. The age and sex distribution of both groups are shown in Fig. 1.

1. Splenic size and weight

The largest length of spleen in the study group was between 5-17cm and the average value was 10.2cm. That of the control group was 7-15.5cm and the average was 10.1cm(Fig. 2). The weight in the study group was between 34.0-246.7gms and the average was 103.6gms and that of the control group was between 42.5-400.9gms and the average was 128.3gms(Fig. 3). Although there was little difference in splenic size and the

splenic weight looked heavier in the control group, the splenic size and weight were increased in the study group compared to the control group (because they are known to decrease with age). Statistically, there was a meaningful difference between the two similar age groups($P < 0.05$).

2. Types of splenic lymphoid change

In order to evaluate the splenic lymphoid change, we used the three types of non-neoplastic proliferation of splenic lymphoid tissue, proposed by Dr. Wolf and Neiman(1989). The first, early activated(EAA) type resembled the white pulp of the immunologically unstimulated state. The second, evolving activated(EVA) type revealed chronic immune stimulation by prominent lymphoid follicles with germinal centers and remarkably wide marginal zones. The third, granulomatous(GRA) type was similar to that seen in other tissues of the body.

By using these criteria, the patients with abdominal neoplasm were divided into three groups(Fig. 4). There were forty six cases(39.0%) of EAA type, seventy one cases(60.2%) of EVA type and one case(0.8%) of mixed GRA & EVA type.

In contrast, the control group showed eighteen

Fig. 2. Comparison of spleen size between abdominal neoplasm & traumatic rupture patients.

Fig. 3. Comparison of spleen weight between abdominal neoplasm & traumatic rupture patients.

EAA : early activated EVA : evolving activated
GREVA : Mixed granulomatous & evolving activated

Fig. 4. Types of splenic lymphoid change in 118 abdominal neoplastic patients.

EAA : early activated EVA : evolving activated

Fig. 5. Types of splenic lymphoid change in 26 traumatic rupture patients.

cases(69.2%) of EAA type, eight cases(30.8%) of EVA type and no case of GRA type(Fig. 5). Between the two groups, there was a statistically significant difference($P < 0.05$).

On microscopic examination, the architecture of the white pulp in the EAA type showed little or no lymphoid follicles, and no distinct mantle or marginal zones(Fig. 6 left) and that in the EVA type showed diffusely follicular hyperplasia with partially or fully formed tripartite germinal centers and widely expanded marginal zone by predominantly medium-sized lymphocytes(Fig. 6 right) characterized by a round, pale staining nucleus with one or two nucleoli and a fair quantity of faintly pyronophilic cytoplasm(Fig. 7). Amorphous PAS-positive intercellular material was sometimes seen with the hyalinized germinal centers of the residual follicles.

Fig. 6. The white pulp of EAA type shows little or no lymphoid follicles(left) and that of EVA type shows follicular hyperplasia with prominent germinal center and marginal zone(right).

Fig. 7. The expanded marginal zone(★) is composed of medium sized lymphocytes with a fair quantity of faintly pyronophilic cytoplasm.

3. Distribution of lymphoreticular cells identified by immunohistochemical stains

There were many more numbers of B and T lymphocytes by positive reaction for L26 & UCHL1(Fig. 7 & 8) in the EVA types of the study group compared to the EAA types of the control

group. In the EVA types of the study group, the increased number of medium sized lymphocytes in the marginal zone of lymphoid follicles exhibited a strong membrane staining for L26 & IgM, a moderate membrane staining for IgG, and were negative for IgD(Fig. 9). There was also an increased number of natural killer cells by Leu7 staining(Fig. 10, left) and a slightly increased number of dendritic cells in the germinal center and perifollicular area by S-100 staining (Fig. 10, right). But the results of macrophage and monocyte markers by lysozyme and ANBE were not meaningful because positive reactions were largely localized in the red pulp and there was not difference between the two groups.

Summarizing the immunohistochemical reaction there were increased numbers of B (especially marginal area), T, natural killer, and dendritic cells especially in EVA types of the study group (Table 2).

Fig. 9. The lymphocytes of marginal zone reveal a moderate membrane staining for IgG(left), strong for IgM(middle) and negative for IgD(right).

Fig. 8. Positive reaction for L26(left) and UCHL1(right) in the EVA type.

Fig. 10. There is an increased number of NK cells by Leu7 staining(left) and dendritic cells by S-100 protein (right) in the EVA type.

Table 2. Comparison of Lymphoreticular Cell Distribution Between EAA & EVA groups

Cell Type/MoAb	LF-B		MgZ-B		T	NK	Mac	Mono	IDC
	L26	IgG/M/D	L26	IgG/M/D	UCHL1	Leu7	Lys	ANB	S100
EAA	+	+/+	+	+/+/-	+	+/-	+/-	UD	F+
EVA	++	+/+/+	++	+/+/-	++	+	+/-	UD	+/-

LF-B : B cells in lymphoid follicles, MgZ-B : B cells in marginal zone,
T : T cells, NK : natural killer cells, Mac : macrophage, Mono : monocytes,
IDC : interdigitating cells, Lys : lysozyme, ANB : alpha-naphthyl butyrate esterase,
UD : undetermined, EAA : early activated type, EVA : evolving activated type

DISCUSSION

During the past two decades, notable advances have been made in understanding of splenic function and fundamental structure. Many factors have contributed to this understanding, including more precise knowledge of the nature of cell membranes and their contents, the availability of radioisotopic methods as well as the use of transmission and scanning electron microscopy (Bishop et al., 1982).

The role of the spleen in the immune function is somewhat speculative and not well documented, but the spleen appears to play a major role in the early antibody response to circulating antigens to which the host has not been previously exposed (Kichen, 1977). The first description of antigen localization in the spleen was presented by White(1963), who investigated antigen trappings in the spleens of chickens and found trapped antigen located along dendritic cell processes of nonlymphoid cells. Rowley(1950) demonstrated that splenectomized patients showed no significant antibody titers following the injection of sheep red blood cells. In contrast, nonsplenectomized control subjects showed a significant rise in antibody titer within 9 to 14 days.

Splenic lymphoid tissue has been known to be affected by many physiologic and pathologic processes but reports about splenic lymphoid change in the patients with neoplasms are rare (Aozasa et al., 1993). Therefore, we tried to observe the influence of the abdominal neoplasms on splenic lymphoid tissue. The abdominal neoplasms in 121 patients included both malignant and benign tumors of the stomach, pancreas, colon, ovary, kidney, gallbladder, and adrenal gland, and the majority of cases (84.3%) were from adenocarcinoma of the stomach.

In splenic size and weight, there was no difference in the average size between the study group(10.2cm) and the control group(10.1cm) but the average weight of the control group(128.3gms) was rather heavier than the study group(103.6gms). However, there was a statistically meaningful difference between the two similar age groups($P < 0.05$), because spleen size and weight have been known to decrease with age.

To analyze the lymphoid response, we observed three major compartments of splenic white pulp; the periarteriolar lymphocyte sheath(PALS), the follicles and the marginal zone, surrounding both PALS and follicles. The splenic nonneoplastic lymphoid

proliferation was divided into three types such as early activated immune reaction(EAA), evolving activated immune reaction(EVA) and granulomatous reaction(GRA), as proposed by Dr. Wolf and Neiman(1989).

The early activated immune reaction, referred to as "immunoblastic hyperplasia" by Burke(1981) and "activated A" by Lukes(1970), is believed to be the early stage of immunologic activation. The white pulp may not be greatly increased in size, and therefore, the spleen may be grossly unremarkable. The evolving activated immune response, termed "activated B" by Lukes(1970), is characteristic of chronic immune response and the spleen may be markedly enlarged with expanded white pulp.

In the present study, there were 46 cases(39.0%) of EAA type among the examined 118 patients with various abdominal neoplasms, and 18 cases(69.2%) among the 26 patients with traumatic rupture. Microscopically, the architecture of the white pulp resembled that of the immunologically unstimulated state because germinal centers were usually not seen and the malphigian follicles lacked distinctive mantle zones. In contrast, there were 71 cases(60.2%) of EVA type in the study group compared to 8 cases(30.8%) in the control group. This pattern was suspected as the result of chronic immune stimulation by blood-borne antigens because there were diffusely follicular hyperplasia with prominent germinal centers and widely expanded marginal zones.

The granulomatous reaction, associated with EVA type, was seen in one case(0.8%) of a patient with stomach leiomyoma. This feature was an isolated incidental finding of little clinical significance.

It has been known that the white pulp of both infancy and senescence may be depleted although the lymphocytes appear stimulated, but that of children and young adults is usually activated due to their frequent exposure to previously unencountered antigens(Wolf et al., 1989). Although the average age of the study group(55 years old) was much older than that of the control group(32 years old), the EVA type was much more common in the study group and the EAA type was common in the control group. There was a statistically significant difference($p < 0.05$) between the two similar age groups.

The anatomic localization of human lymphoreticular cells(B, T cells, natural killer cells, macrophages, and dendritic cells) was studied in an avidin-biotin

complex method by a series of monoclonal antibodies, such as L26, IgG, IgM and IgD for B lymphocytes, UCHL1 for T lymphocytes, Leu7 for natural killer cells, lysozyme for monocytes, and S-100 protein for dendritic cells.

In this study, there were much more numbers of B and T lymphocytes by positive reaction for L26 and UCHL1 (Fig. 7) in the EVA types of the study group compared to the EAA types of the control group. T cells were mainly present in the central area of PALS and B cells in the peripheral part of the PALS, follicles and marginal zone. From flow cytometric studies (Ewijk et al., 1985) the spleen contains 40-50% B cells and 25% T cells. In the EVA types of the study group, the increased number of medium sized lymphocytes in the marginal zone exhibited a strong membrane staining for L26 and IgM, a moderate membrane staining for IgG, and were negative for IgD (Fig. 8). These findings were quite similar to those of previous reports (Ewijk et al., 1985; Eikelenboom et al., 1985; Timens et al., 1985). MacLennan et al. (1982) proposed that the marginal zone was a distinct anatomic lymphocyte compartment in the spleen with unique immunohistologic features and that at least an (IgD—) part of the marginal zone cells was a distinct functional lineage of nonrecirculating B cells. There was also an increased number of natural killer cells and a slightly increased number of dendritic cells in the germinal center and perifollicular area in the EVA types of the study group. But the results from macrophages and monocytes by lysozyme and alpha-naphthyl butyrate esterase were hard to interpret because large numbers of leukocytes were admixed and the frozen section for ANBE was not good to examine in detail.

In vitro studies (Eikelenboom et al., 1985) showed that both T and B lymphocytes as well as mononuclear phagocytes are essential for most types of immune responses, and the interaction among antigen, T lymphocytes, B lymphocytes and mononuclear phagocytes takes place within a framework of reticular cells in the peripheral lymphoid organs. In addition, there were reports that the relative absence of Leu7+ cells was correlated with the low natural killer cell activity in newborns (Timens et al., 1987) and that depletion of macrophages in the spleen caused a strong decrease in the numbers of antibody-forming cells after intravenous injection of the antigen (Deleamarre et al., 1990).

Based on the findings of increase numbers of B

(especially marginal area), T, natural killer and dendritic cells especially in the EVA types of the study group, we assume that the splenic lymphoid tissue in the various kinds of abdominal neoplasms, mostly malignant, is activated in the chronic immune state characterized by prominent lymphoid follicles with germinal centers and distinct marginal zone by some kinds of unknown blood-borne antigens.

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