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Expression of Interleukin-6 in Polymorphic Reticulosis
—Immunohistochemical study of 5 cases—

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Peripheral T cell lymphoma encompasses lymphomas with a variety of histologic appearances and clinical patterns. Recently, it has been suggested that almost all of the histologic features described under the name of polymorphic reticulosis(PR), lethal midline granuloma, and midline malignant reticulosis can be included in those generally described for malignant lymphomas of peripheral T cell origin(PTCL). There have been few studies of pathogenesis or tissue damage mechanism in PR patients. The need for a precise mechanism for tissue damage has important therapeutic implications. Using immunohistochemical methods with polyclonal anti IL-6 antibody, the authors describe 5 cases of PR with clinically and pathologically typical PR demonstrating a high expression of IL-6. According to classification, 2 cases of grade 1 PR showed the highest expressions, and 2 cases of grade 2 PR with atypical lymphoid cells showed moderate activity, but one case progressed into frank lymphoma(grade 3) and lost IL-6 expression.
This strongly implies that some cases of PR have a different mechanism of tissue damage from frank PTCL, despite the one disease spectrum. Further studies on more cases may help clarify the pathogenesis.

Key Words : *Peripheral T cell lymphoma, Interleukin-6, Polymorphic reticulosis*

INTRODUCTION

Polymorphic reticulosis(PR), also known as midline malignant reticulosis, is a term to describe aggressive lymphoreticular lesions involving the upper aerodigestive tract or midline facial structures. The histologic features of PR are identical to those of sinonasal "angiocentric immunoproliferative lesions(AIL)", which are characterized by angiocentric and angioinvasive lymphoid infiltrates with a wide range of cytologic atypia(Stricher et al., 1994). In recognition of their common features, a grading scheme was proposed to include all cases of AIL, ranging from lymphocytic vasculitis to angiocentric lymphoma. Moreover, the grading scheme was shown to be clinically useful in one large retrospective series(Lipford et al., 1988). Although infectious and immune mechanisms have been proposed, many investigators postulate that PR is a non-Hodgkin's lymphoma.

But studies about the pathogenetic mechanism of PR are lacking, although recent studies show that the lymphoid cells in PR may contain Epstein-Barr virus(EBV) genomes or antigens, suggesting an infectious component to the pathogenesis. Moreover, cli-

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nical studies are small, incorporate variable diagnostic and staging criteria, use various pathologic criteria and approach therapy differently.

Interleukin-6(IL-6) is a pleiotrophic cytokine that exerts many biologic effects, including both growth and differentiation inducing activities(Kishimoto, 1989). IL-6 can induce B cell differentiation, augment plasmacytoma growth, and induce proliferation of cytotoxic T cells as well as of early precursors of the hematopoietic compartment (Wolvekamp and Marquet, 1990 ; Kishimoto, 1992). This cytokine is produced by several types of cells, including monocytes, macrophages, granulocytes, fibroblasts, endothelial cells, epidermal cells, lymphocytes activated in vitro and various tumor cells(Gauldie et al., 1987 ; Helle et al., 1988 ; Houssian et al., 1988 ; Kawano et al., 1988 ; Jourdan et al., 1990). Excess production of IL—6 may be involved in the pathogenesis of several diseases, including Casthleman s disease and Kaposi s sarcoma(Kishimoto, 1989 ; Nachbauer et al., 1991 ; Macon et al., 1992 ; Muller et al., 1992).

In this study we examined a possible role of IL-6 in 5 cases of polymorphic reticulosis, including one case of developed conventional ML. We performed immunohistochemical staining for IL-6 on 4 cases of PR with a case of frank PTCL, and compared and discussed the results of IL-6 expression.

MATERIALS AND METHODS

Surgical specimens of biopsies from PR patients diagnosed at Ewha Womans University Hospital during 1993-1994 were obtained. Three specimens were nasal cavity tissue and one case was tonsillar tissue. The previous biopsy diagnoses were made as grade 1 PR in two cases and PR with atypical cells infiltration(grade 2 PR) in two cases. Among them one case developed frank PTCL 6 months later. The clinical findings are summarized in Table 1(Table 1). We chose the blocks which represent cellular area without necrosis. As a control tissue, non-neoplastic tonsillar tissue was used. Specimens were routinely fixed with 10 % buffered formalin and processed. Brief patient histories were summarized in Table 1.

Antibody preparation ; Anti-IL-6 antibodies were a generous gift from KIST(Korea Institute of Science and Technology). In brief, rabbits were immunized with human recombinant IL-6. 250 ug of IL-6 in 2.25 ml of PBS was emulsified with 500 ul of Imject Alum. Each rabbit received four subcutaneous injections(500 ul each) of the IL-6/Alum suspension at the back of the animal and adjacent to each of these sites, four 500 ul injections of complete Freund's adjuvant/PBS subcutaneously. And then, each rabbit received three booster injection of 50 ug of IL-6/Alum, adjacent injections with incomplete Freund's adjuvant emulsi fied in PBS, and additionally 5 ug of IL-6 intravenously. Every 50 ml of blood were aspirated, coagulated, decomplemented at 56 C, and stored at —70℃. The neutralizing capacity was 300 ug of IL-6 per ml of the pooled antiserum. The anti IL-6 antibodies showed no neutralizing reactivity against the other IL-1, IL-2, IL-4 and TNF.

Immunohistochemistry ; Previously described immunohistochemical staining procedures were used for expression of IL-6 (Hsu et al., 1981). Briefly, serial 5 um sections were cut from 10 % formalin fixed and paraffin embedded tissue. After deparaffinization and hydration, sections were incubated for 20 min with 10 % normal goat serum to block nonspecific serum protein binding. This was followed by a block for endogenous peroxidase activity using 3 % hydrogen peroxide for 15 min. Sections were then incubated with anti IL-6 antisera and control sections were

Table 1. Summary of clinical findings.

Case No.	Age	sex	site	Dx.
#1	43	M	tonsil	PR(AIL, grade 1)@
#2	52	M	Nasal cavity	PR(AIL, grade 1)
#3	46	F	Nasal cavity	PR with ALC(AIL, gr 2)*
#4	48	M	Nasal cavity	PR with ALC(AIL, gr 2)
#5	46	F		PTCL(AIL, gr 3)

PR with ALC:polymorphic reculosis with atypical lymphoid cells ; gr : grade ; AIL : angiocentric immunoproliferative lesion ; PTCL : peripheral T cell lymphoma.

@ Grading system(Lipford et al., 1988)

* Frank malignant lymphoma(AIL, grade 3) was developed, later(case #5).

incubated with phosphate-buffered saline(PBS). After 2 hours incubation, sections were washed three times in PBS and incubated with universal secondary antibody(Dako Co. Calif.) for 10 min. After which they were incubated with avidin-biotin reagents from a LSAB kit. All sections were reacted with AEC as the chromogen. The staining methods for phenotypic and IL-6 expression were the same. All monoclonal antibodies and staining kits(LSAB) used in this study were obtained from Dako(Calif., U.S.A)

RESULTS

1) Light microscopic findings and phenotypic expression

The biopsies of cases #1 and #2 showed the polymorphic infiltration of lymphocytes, histiocytes and a few atypical cells, accompanied by mucosal destruction and severe necrosis. The atypical cells were aggregated in blood vessels. The diagnosis was made as polymorphic reticulosis(AIL, grade 1). Histologic examination of nasal cavity and tonsillar mass reveals typical polymorphic reticulosis.

Cases #3 and #4 revealed similar histopathology except for more and diffuse atypical cells infiltration. The atypical cells were over fifty percent. The diagnosis was made as PR with atypical cells infiltration(AIL, grade 2). Case #3 developed frank peripheral T cell lymphoma with most atypical lymphoid cells, later(AIL, grade 3), and designated as "case #5". She got remission with radiotherapy of 3000 rad dose, but relapsed after 6 onths later. The later biopsy revealed the worse histopathology, AIL, grade 3. She is well now with second remission by chemotherapy.

The phenotypes of atypical cells from all 5 cases were T cell. Most large atypical neoplastic cells were positive for MT-1, UCHL-1, and negative for L-26, and CD 68.

All four patients are lived well without disease recurrence till now.

2) Immunohistochemical findings for IL-6.

IL-6 on nonneoplastic tonsillar tissue was negative. In cases #1, #2, the immunohistochemistry for IL-6 on previous biopsy specimens revealed diffuse and strong positivity in polymorphic cells, including lymphocytes, histiocytes, endothelial cells, and reactive plasma cells(Fig.1). The epithelium also showed focal and weak positivity. In cases #3 and #4, IL-6 showed moderate positivity in endothelial cells and many lymphocytes(Fig. 2). Staining on a later biopsy-(case #5), which was frank PTCL, showed that there was no expression of IL-6 except for focal and weak

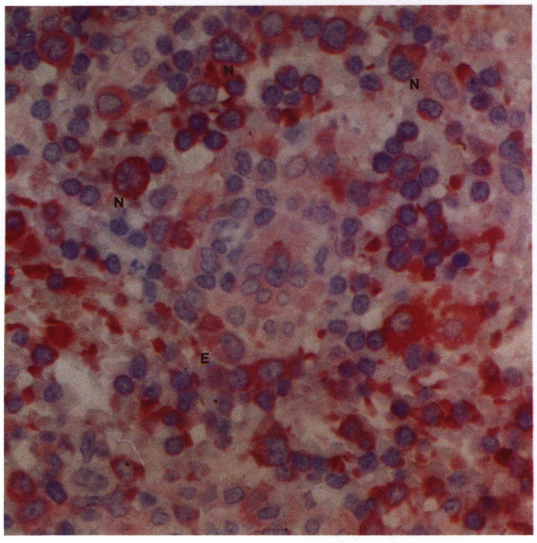

Fig. 1. Polymorphic neoplastic cells(N) and endothelial cells(E) show strong reactivity for IL-6 in PR(AIL, grade 1) of Case #2 (Immunostain for IL-6, × 100).

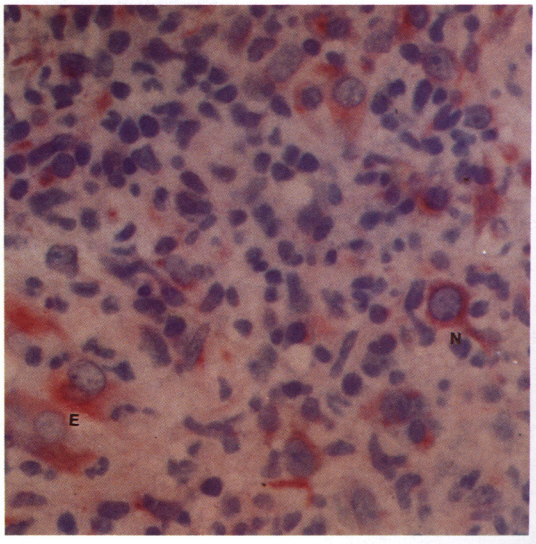

Fig. 2. Some neoplastic cells(N) and endothelial cells(E) reveal focal and weak positivity for IL-6 in PR with atypical lymphoid cells(AIL, grade 2) in Case #3 (Immunostain with ABC method for IL-6, ×100).

staining of endothelial cells. Atypical tumor cells were totally negative(Fig. 3).

DISCUSSION

Interleukin-6 is a multifunctional cytokine that regulates immune response, acute phase reactions and hematopoiesis. Deregulated IL-6 gene expression has been implicated as being involved in the pathogenesis of a number of diseases, especially autoimmune diseases and plasma cell neoplasias. The detection of autocrine or paracrine cytokine production in some B cell lymphomas may be an additional ground for the hypothesis of a continuous neoplastic cell growth (Hutchins et al., 1990 ; Echmann et al., 1992 ; Puri and Leland, 1992). Several studies of cytokine expression in Hodgkin s and T cell lymphoma indicated that lymphoma-specific cytokine expression which may not only be responsible for defined clinical symptoms but also influence the individual cellular composition of malignant lymphomas and their growth behavior(Merz et al., 1991 ; Tesch et al., 1992 ; Hsu et al., 1993).

Lethal midline granuloma is a clinical syndrome rather than a specific entity. The term refers to the presence of a destructive lesion of the upper respiratory tract, including the nose, nasopharynx, palate, paranasal sinuses, and midface. It encompasses at least three different pathologic conditions. Some of these cases represent particularly aggressive examples of Wegener's granulomatosis. Others have the microscopic appearance of a conventional malignant lymphoma, usually of large cell type. Still others exhibit a polymorphic microscopic appearance unlike that of any conventional lymphoma ; they have been designated as polymorphic reticulosis, malignant histiocytosis, and midline malignant reticulosis. In this form, small lymphocytes alternate with large atypical cells. According to Fechner and Lamppin, it is the polymorphism that is crucial for its distinction from a conventional lymphoma(Fechner and Lamppin, 1972). In some of these patients the disease remains localized ; in an approximately equal number, dissemination occurs.

There have been few studies about the extensive tissue damage mechanism of polymorphic reticulosis(Emillei et al., 1992). In the present study, we performed an immunohistochemical study of IL-6, and we noticed that many polymorphic lymphoid cells as well as plasma cells expressed IL-6 activity. Of 5 cases studied, two cases of PR(AIL, grade 1) expressed higher levels of IL-6 than 2 cases of PR with atypical cells infiltration(AIL, grade 2). One case developed to ML with the most atypical lymphoma cells(AIL, grade 3), IL-6 expression was markedly increased in low grade PR but there was no expression in high grade.

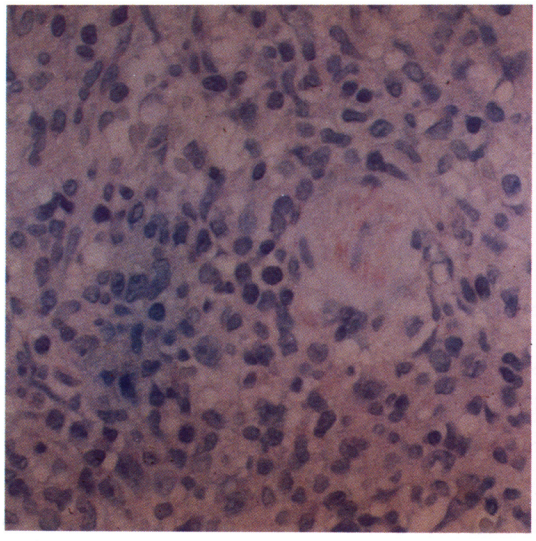

Fig. 3. There is a marked decrease of IL-6 expression in PTCL (AIL, grade 3) which developed from Case #3 (Immunostain for IL-6, × 100).

So we could conclude that IL-6 may play a role in tissue damage such as tissue destruction, extensive necrosis and autocrine effect on neoplastic proliferation in the early phase of disease, considering the aforementioned biologic effects of IL-6. Because we studied a few cases, the finding that frank PTCL did not show IL-6 expression needs further studies on more cases.

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