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Human Fibroblasts In Idiopathic Retroperitoneal Fibrosis Express HLA-DR Antigens

Inchul Lee, M. D.

Department of Pathology Asan Medical Center College of Medicine, Ulsan University Seoul 138-040, Korea

Idiopathic retroperitoneal fibrosis (IRF) is a rare human disease characterized by non-neoplastic fibroblastic proliferation associated with chronic inflammatory cells; its pathogenesis is obscure. We undertook an immunohistochemical study for the expression of HLA-DR antigens and other immune-related markers by retroperitoneal proliferating fibroblasts and inflammatory cells from 2 IRF patients. Patterns of immunoreactivity were compared with those expressed by human nodular fasciitis (NF) and granulation tissue. In IRF, most fibroblasts immunostained strongly for HLA-DR antigens, whereas fibroblasts in NF and granulation tissue did, not immunostain at all. The fibroblasts did not immunostain for interleukin 2 receptor, C3b receptor, CD-4, CD-8, or Leu-M1 in any of the tissue studied. Most macrophages and lymphocytes in IRF and NF immunostained Strangly for HLA-DR antigens. In IRF, the CD-4 and CD-8 immunostained T-lymphocytes appeared equally distributed. The expression of HLA-DR antigens by fibroblasts in IRF indicates that this rare disease may indeed be an immune-associated hypersensitivity disorder.

Key Words : *Idiopathic retroperitoneal fibrosis, HLA-DR antigens*

INTRODUCTION

In IRF patients, the retroperitoneum is expanded by proliferating fibrous tissue, which, by constricting and finally obliterating the ureters, results in progressive renal failure (Leport and Walsh, 1979). There are also, albeit very rare, localized forms of the process involving the periureteral or renal pelvic regions (Harbrect, 1967). IRF may be associated with a similar process in the mediastinum, sclerosing cholangitis, Riedel's thyroiditis, pseudotumor of the orbit, or generalized vasculitis (Comings et al., 1967 : Hellstrom and Perez-Stable, 1966).

The retroperitoneal fibrous tissue consisted of proliferating fibroblasts with collagen deposition and a prominent mixed inflammatory infiltrate comprised of lymphocytes, plasma cells, macrophages, and eosinophils. Lymphoid follicles with germinal centers were frequently present.

The pathogenesis of IRF is not known. However, the mixed inflammatory infiltrate with lymphoid follicles suggests that IRF may be an immune-associated hypersensitivity disorder. In favor of this notion, there have been several cases reported to be secondary to the administration of methysergide and other durgs, with occasional dramatic regression of the disorder after cessation of theraphy(Graham et al., 1966). In order to improve our understanding of the pathogenesis of IRF, we undertook an immunohistochemical study for the expression of HLA-DR antigens and other immunerelated markers by retroperitoneal fibrous tissues from 2 IRF patients.

Address correspondence to : *Dr.Inchul Lee, Department of Pathology Asan Medical Center College of Medicine, 388-1 Poongnap-Dong, Songpa-Ku Seoul 138-040 Korea Tel (02)480-3312*

MATERIALS AND METHODS

Tissue samples of IRF were obtained from 2 male patients. A 50-year-old man had IRF with diffuse involvement of the retro-peritoneum and subsequent obstructive renal failure;he underwent surgery to relieve the urinary obstruction. The second patient was a 68-year-old man who had a localized lesion in a kidney which led to nephrectomy. Tissue samples were frozen promptly after surgery in liquid nitrogen and kept at −70C until used. Cryostat sections were prepared and immunostained with commercially available monoclonal antibodies:HLA−DR, interleukin 2 receptor, C3b receptor (Dako Corportion, Santa Barbara, CA), CD−4, CD−8 and Leu−M1 (Becton Dickinson Co., Mountain View, CA). The HLA−DR monoclonal antibody is known to immunoreact with an antigenic determinant present on the beta-chain of all HLA−DR molecules (Ziegler et al., 1982). Immunostaining was accomplished with the avidin−biotin complex method (Hsu et al., 1981) and indirect immunofluorescence. As positive controls, cryostat sections of a reactive lymph node were similary stained. Negative controls were performed by omitting the primary antibody and substituting nonimmmune serum.

For comparison, samples from 2 cases of typical nodular fasciitis (NF) and from surgically−resected granulation tissues were similarly studied. One NF patient was a 39−year−old man who had a lesion in the left leg; the second patient was a 32−year−old female with a lesion in the chest wall.

RESULTS

The IRF samples displayed the typical features of fibroblastic proliferation with collagen deposition, prominent inflammatory infiltrates, and occasional lymphoid follicles (Fig. 1). The inflammatory infiltrate consisted of lymphocytes, macrophages, plasma cells, and eosinophils. Retroperitoneal lymph nodes in the case of diffuse IRF also contained an inflammatory infiltrate similar to that in the retroperitioneal tissue. The NF samples displayed the characteristic fibroblastic proliferation in a myxoid stroma, Inflammatory infiltrates were focally present;however, no lymphoid follicle formation was noted. The sample of granulation tissue consisted of fibroblasts, numerous capillaries, and chronic inflammatory infiltrates.

By immunohistochemistry, most of the fibroblasts in the IRF's immunostained for HLA−DR antigens (Fig. 2a & b), whereas they were not immunostained in the sample of NF or in the granulation tissue (Fig. 3a & b). Although the HLA−DR immunostained fibroblasts were diffusely distributed, they were more frequently and intensely immunostained in the vicinity of the lymphoid follicles and inflammatory cells. In neither the IRF nor the NF were fibroblasts immunostained with interleukin 2 receptor, C3b receptor, CD−4, CD−8, or Leu−M1 antibodies. In both IRF and NF, many mononuclear inflammatory cells strongly immunostained for HLA−DR antigens and Leu−M1 (Hsu and Jaffe, 1984). Both cases of IRF displayed similar distribution of CD−4 and CD−

Table 1. Clinical features of each case of idiopathic retroperitoneal fibrosis and nodular fasciitis

Case No.	Patients	Diagnosis	Location	Size (Cm)
1	50M	RF, diffuse	Retroperitoneum	20x12x4
2	68M	RF, localized	Left renal plevis	8x7x5
3	32M	NF	Chest Wall	1.5x1x1
4	39M	NF	Left lower leg	2x1.5x1.2

RF : retroperitoneal fibrosis
NF : nodular fasciitis

Fig. 1. IRF: Note the proliferating fibroblasts admixed with a mixed inflammatory infiltrate and a lymphoid follicle (LF). (H&Ex100)

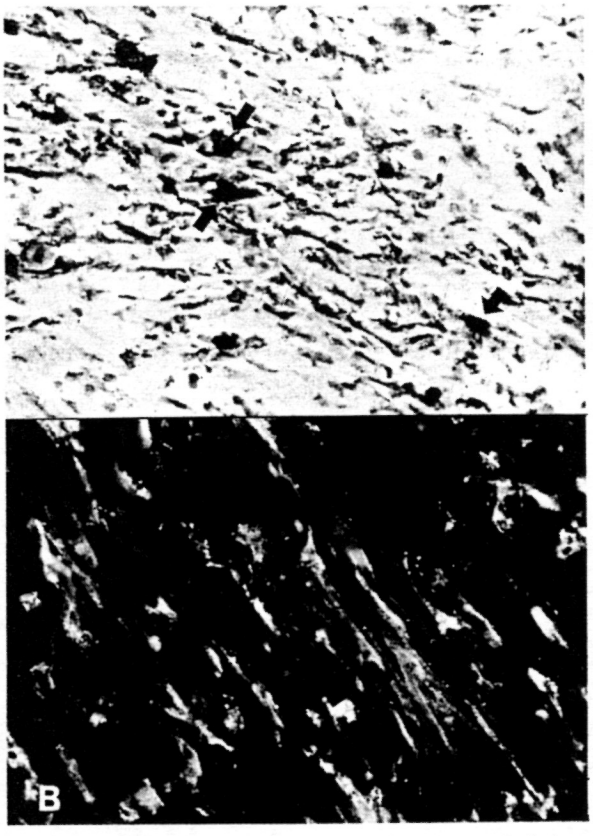

Fig. 2a. IRF: Note the strong immunostaining for HLA−DR in the fibroblasts (spindlecells) as well as macrophages. (ABC method, x250)
Fig. 2b. IRF (same case as 2a): Fibroblasts dliffusely immunostained for HLA−DR. Note fine granular pattern of immunostaining (Immunofluorescence. x450)

Fig. 3a. NF: Macrophages display strong immunostaining for HLA−DR whereas fibroblasts are not immunostained. (ABC method, x250)
Fig. 3b. NF (same case as 3a): Only macrophages are immunostained for HLA−DR. No spindle cell (fibroblast) immunostained. Macrophages display granular pattern of immunostaining (Immunofluorescence, x 450)

8 immunostained T−lymphocytes. Eosinophils and lymphocytes were occasionally stained with anti−interleukin 2 receptor antibody. Lymphocytes in the germinal centers and macrophages strongly immunostained for C3b receptor as previously describe (Gerdes et al., 1982).

DISCUSSION

Fibroblasts in IRF immunostained for HLA−DR antigens, whereas fibroblasts in NF or granulation tissue did not. IRF is a slowly progressive condition; based on its morphologic features and certain clinical observations, it has been suggested that it may be

an immune-associated hypersensitivity disorder. NF is a rapid, localized proliferation of fibroconnective tissue and vessels (Enzinger and Weiss, 1983). NF mimicks malignant soft tissue sarcomas;yet, the proliferative process is self-limiting and usually results in complete healing in several months. Inflammatory infiltrates in NF are less prominent than those in IRF, and lymphoid follicles are absent. The pathogenesis of NF is not clear. However, given its natural history and the comparative paucity of inflammatory cells, NF has not been regarded as an immune-associated hypersensitivity disorder and may thus be viewed as a "negative" control for IRF.

HLA-DR antigens, the human counterparts of mouse Ia antigens, are polymorphic cell surface glycoproteins involved in the initiation of the immune response (for reviews see Behacerraf, 1981; Kaufman et al., 1984). HLA-DR antigens are normally restricted to "antigen-presenting" cells such as B Lymphocytes, macrophages, dendritic cells, Langerhans cells (Rowden et al., 1977) and some endothelial cells (Hlrschberg et al., 1980). Renal tubular epithelial cells may also express low levels of HLA-DR antigens (Fuggle et al., 1983). Under certain conditions, other human epithelial cells may also be induced to express HLA-DR antigens as examplfied by thyroid follicular cells in Grave's disease and Hashimoto's thyroiditis (Aichinger et al., 1985;Hanafusa et al., 1983), and by keratinocytes in graft-vs-host reaction (Lampert et al., 1981). Normal fibroblasts are thought to lack HLA-DR antigensin vivo. It was recently reported that some neoplastic "fibroblasts" in malignant fibrous histiocytomas may express HLA-DR antigens (Roholl et al., 1985); yet, the nature of those neoplastic "fibroblasts" was not clear. To date, there has been no convincing in vivo demonstration of HLA-DR expression by nonneoplastic human fibroblasts;this study, however, shows that distinctly non-neoplastic fibroblasts such as those of IRF may express HLA-DR antigens in vivo.

The observation of HLA-DR antigenic expression by fibroblasts in IRF provides suggestive evidence that this disease may indeed have an immune-related pathogenesis. Given their demonstrated HLA-DR expression, it may be further speculated that fibroblasts may have activated T lymphocytes by presenting certain antigens, either acquired or autoimmune. By secreting T cell lymphokines (Scher et al., 1980), the activiated T cells, in turn, might have induced HLA-DR expression by additional fibroblasts and thus initiated a "vicious cycle" of an immune-associated hypersensitivity disorder.

While it is possible that not every cell expressing HLA-DR antigens may be capable of processing and presenting antigens, there have been reports that cultured mouse and human fibroblasts may function as immune accessory cells in vitro (Habu and Raff, 1977;Katz and Unanue, 1973;Lipsky and Kettman, 1982). It was also recently reported that cultured mouse fibroblasts transfected with human HLA class II genes may express the corresponding class II HLA antigens and may activate T-lymphocyte clones by presenting certain antigens (Austin et. al., 1985;Malissen et al., 1984). Considering that cultured mouse embryo fibroblasts may bind sufficiently haptenated proteins to stimulate secondary anti-hapten antibody responses in vitro (Katz and Unanue, 1973), we may further postulate that in drug-related IRF the drugs may serve as haptens which may be presented to helper T-lymphocytes by the HLA-DR expressing fibroblasts. Our findings suggest that IRF may indeed be an immune-associated hypersinsitivity disorder;nevertheless, the triggering mechanism for the fibroblastic proliferation in IRF remains unclear.

REFERENCES

Aichinger G, Fill H, Wicck G : *In situ immune complexes, lymphocyte subpopulations, and HLA-DR-positive epithelial cells in Hashimoto thryoditis. Lab Invest 52;132-140, 1985.*

Austin P, Trowsdale J, Rudd C, Bodmer W,

Feldmann M, Lamb J:*Functional expression of HLA−DP genese transfected into mouse fibroblasts. Nature 313;61−64, 1985.*

Benacerraf B:*Role of MHC gene products in immune regulation. Science 212; 1229−1238, 1981.*

Comings DE, Skubi KB, van Eyes J, Motulsky AG: *Familial multifocal fibrosclerosis. Findings suggesting that retroperitioneal fibrosis, mediastinal fibrosis, sclerosing cholangitis, Riedel's thyroiditis, and pseudo−tumor of the orbit may be different manifestations of a single disease. Ann Intern Med 66;884−892, 1967.*

Enzinger FM, Weiss SW:*Nodular fasciitis. PP 15−24, In soft tissue tumors. C.V.Mosby Co. 1983.*

Fuggle SV, Errasti P, Daar AS, Fabre JW, Ting A, Morros PJ:*Localization of Major histocompatibility complex (HLA−ABC and DR) antigens in 46 kidneys. Differences in HLA−DR staining of tubules among kidneys. Transplantation 35;385−390, 1983.*

Graham JR, Suby HI, Lecompte PR, Sadowsky NL:*Fibrotic disorders associated with methysergide therapy for headache. N Engl J Med 274;359−368, 1966.*

Hanafusa T, Pujol−Borrell R, Chiovato L, Russe11 RCG, Doniach D, Bottazzo GF:*aberrant expression of HLA−DR antigen on thyrocytes in Grave's disease: Relevance for autoimmunity. Lancet 2:1111−1115, 1983.*

Habu S, Raff MC:*Accessory cell dependence of lectin−induced proliferation of mouse T lymphocytes. Eur J Immunol 7;451−457, 1977.*

Harbrecht PJ:*Variants of retroperitoneas fibrosis. Ann Surg 164;388−401, 1967 Hirschberg H, Bergh OJ, Thornsby E:Antigen presenting properties of human vascular endothelial cells. J Exp Med 152;249s−225s, 1980.*

Hellstrom HR, Perez-Stable ED:*Retroperitoneal fibrosis with disseminated vasculitis and intrahepatic sclerosing cholangitis. Am J Med 40;184−187, 1966.*

Hsu SM, Raine L, Fanger H:*The use of avidin −biotin peroxidase complex (ABC) in immunoperoxidase techniques:A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29;577−581, 1981.*

Hsu SM, Jaffe ES:*Leu M1 and Peanut agglutinin stain the neoplastic cells of Hodgkin's disease. Am J Clin Pathol 82;29−32, 1984.*

Katz DH, Unanue ER:*Critical role of determinant presentation in the induction of specific responses in immunocompetent lymphocytes. J Exp Med 137;967−990, 1973.*

Kaufman JF, Auffray C, Korman AJ, Shackelford DA, Strominger J:*The class II molecules of the human and murine major histocompatibility complex. Cell 36;1−13, 1984.*

Lampert IA, Suitter AJ, Chisholm:*Expression of Ia antigen on epidermal Keratinocytes in graft-vs-host disease. Nature 293;149−150,1981.*

Lepor H, Walsh PC:*Idiopathic retroperitoneal fibrosis. J Urol 122;1−6, 1979.*

Lipsky PE, Kettman JR: *Accessory cells unrelated to mononuclear phagocytes and not of bone origin. Immunol Today 3:36−42, 1982.*

Malissen B, Peele Price M, Govèrman JM, McMillan M, White J, Kappler J, Marrack P, Pierres A, Pierres M, Hood L:*Gene transfer of H−2 class II Genes:Antigen presentation by mouse fibroblast and Hamster B−cell lines. Cell 36;319−327, 1984.*

Roholl PJM, Kleyne J, Van Unnik JAM: *Characterization of tumor cells in malignant fibrous histiocytomas and other soft−tissue tumors, in comparison with malignant histiocytes. II. Immunoperoxidase study on cryostat sections. Am J Pathol 121:269−274, 1985.*

Rowden G, Lewis, Sullivan AK:*Ia antigen expression on human epidermal Langerhans cells. Nature 268−274, 1985.*

Scher MG, Beller DI, Unanue ER:*Demonstration of a soluble mediator that induces exudates rich in Ia−positive macrophages. J Exp Med 152;1684−1690, 1980.*

Ziegler a, Uchanska-ziegler B, Zeuthen J, Wernert P:*HLA antigen expression at the single cell level on a K652xB cell hybrid:An analysis with monoclonal antibodies using bacterial binding assays. Somatic Cell Genet 8;775−789, 1982.*