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Rapid Communication

Tumor Necrosis Factor Receptors in Lymphoid Tissues and Lymphomas

Source and Site of Action of Tumor Necrosis Factor Alpha

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Tumor necrosis factor alpha (TNFα), which is produced by germinal center dendritic reticulum cells (DRC) in lymphoid tissue, plays a regulatory role in a local immune response. However no information is available on the nature and location of cells responding to this cytokine. Thus TNF receptor distribution was investigated **in situ** ***by immunohistochemistry using monoclonal antibodies directed against the p75 and p55 receptor proteins. Receptor expression was unique and restricted to the lymphoreticular tissue. The p75 receptor was found on activated lymphocytes and interdigitating reticulum cells of the T-cell area, whereas the p55 receptor was confined to the germinal center DRCs, which are the main site of TNFα production. The two receptor proteins were expressed on distinct cell populations of the lymphoid system and no coexpression was observed. Preliminary results indicate that TNF receptor (TNFR) expression is regulated; Upregulation of TNFR proteins was found in reactive hyperplasia together with increased TNFα expression. In lymphoproliferative disorders, expression of the p75 receptor and TNFα was found mainly in high-grade malignant non-Hodgkin lymphomas. In summary, TNFα produced by germinal center DRCs might regulate an*** **in vivo** ***immune response through autocrine and paracrine pathways. Thus TNFα might signal, through the distinct TNFR proteins, the p55 and p75 receptor, which are expressed on different cell types in lymphoid tissue. (Am J Pathol 1991, 139:7–15)***

Tumor necrosis factor (TNF) is a pleiotropic cytokine that plays an important role in the host defense to infection and in inflammation; antitumor activity is found with certain transplanted murine tumors (for review see Beutler and Cerami[1] and Paul and Ruddle[2]). The precise functional significance of TNF and its role in various diseases are still areas of intensive research. Tumor necrosis factor stands for two homologous peptide factors,[3] TNFα, which is produced by activated monocytes, macrophages, and activated T lymphocytes,[4,5] and TNFβ which is derived from a subpopulation of activated T cells.[6] Tumor necrosis factor exerts its biologic activity by binding to two types of cellular TNF receptors (TNFR).[7,8] Recent immunohistochemical investigations on the location of TNFα production identified the germinal center dendritic reticulum cells (DRC) as the main source of TNFα in tissues.[9,10] *In vitro* studies suggest ubiquitous occurrence of TNFR, and TNF has been shown to exert important effects on lymphocytes,[11–13] monocytes,[14] fibroblasts, and on the endothelium.[15,16]

Because TNFα was shown to be a locally active cytokine with immunoregulatory properties,[11–14] we asked the question how DRC-derived TNFα exerts its effects on the immune tissue *in situ*. It was a reasonable assumption that the TNFα-producing DRCs are unlikely to be the only cells responding to this cytokine. Thus both autocrine and paracrine pathways might be operative for TNFα action. To identify the cellular targets of TNFα, the expression of the two TNFR proteins was investigated *in situ* in lymphoid tissues. Both types of TNFR recently have been characterized by molecular cloning and have been shown to differ by apparent molecular mass (75 and 55 kd) and by their relative expression level on hu-

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man cell lines.[17–20] Monoclonal antibodies (MAbs) have been developed against the p75 TNFR (utr series) and the p55 TNFR (htr series) and shown to be specific and non–cross-reactive,[8] despite the high degree of sequence similarity in the extracellular region from both types of TNFR.[20]

We selected two MAbs suitable for immunohistochemistry, utr-1, a TNF-blocking anti-p75 TNFR antibody, and htr-19, a nonblocking anti-p55 TNFR antibody for the analysis of receptor distribution on frozen sections of normal lymphoid tissues. Receptor expression was correlated with sites of TNFα production. The present immunohistochemical study shows a unique distribution pattern with an exclusive expression of TNFR proteins in lymphoid tissues. The more abundant TNFR protein, p75, was essentially expressed in cells of the interfollicular area and comprised interdigitating reticulum cells and activated lymphocytes. The p55 protein was found in germinal center DRCs, the site of TNFα production. Thus it is postulated that TNFα regulates an immune response through autocrine and paracrine pathways that use different receptor proteins. Furthermore preliminary data obtained from cases with lymphoproliferative disorders suggest that the p75 TNFR expression, together with TNFα, are associated with high-grade malignancy.

Materials and Methods

Tissue and Cell Preparation

Fresh tissues removed at surgery for diagnostic purposes were snap frozen in liquid nitrogen and stored until use at −70°C. Cryostat sections were mounted on gelatine or polylysine-coated glass slides, air dryed for 3 hours, fixed in either 2% paraformaldehyde-lysine sodium periodate or in acetone (only for anti-TNFα antibody incubation) for 10 minutes at room temperature, and rinsed twice in TRIS buffer. Fresh bone marrow cells and peripheral blood leukocytes were obtained from healthy volunteers, isolated on a Ficoll gradient, and suspended in Roswell Park Memorial Institute 1640 medium containing 10% fetal calf serum, 100 μ/ml penicillin, and 100 μg/ml streptomycin. Lymphocytes (10^6/ml) were stimulated either with 1 μg/ml phytohemagglutinin or with 10 μg/ml lipopolysaccharide from *Escherichia* coli (Sigma Chemical Co., St. Louis, MO) for 20 hours at 37°C. Thereafter cells were washed and cytocentrifuged. Cytospin preparations were stored at −80°C until immunostaining.

Immunostaining

Serial cryostat sections were incubated for 30 minutes at room temperature sequentially with the following MAbs: utr-1 (hybridoma supernatant, 1:50 dilution), htr-19 (hybridoma supernatant, undiluted), or an anti-TNFα MAb (1:200 dilution). After two 5-minute washes in TRIS buffer, the sections were incubated with rabbit anti-mouse immunoglobulin (Dakopatts, Glostrup, Denmark; 1:30 diluted) for 30 minutes, followed after rinsing by incubation with alkaline phosphatase anti-alkaline phosphatase (APAAP) complex for 30 minutes thereafter by the addition of the substrate for 30 minutes according to the instructions of the manufacturer (Dalopatts). The immunostaining procedure for the cytospin preparation was identical; the smears were fixed in acetone for 10 minutes at room temperature.

Double Staining

Cryostat sections from utr-1–positive tissues were stained serially for utr-1 and examined by a second staining to analyze the phenotype of TNFR-positive cells. Briefly, utr-1 staining was performed as above; sections were then stained for cell markers (Tac, T11, DRC1, T4, T8, T6, L26) and TNFα using murine monoclonal antibodies and immunoperoxydase developed in 0.02% 3, 3-diaminobenzidine. As control, either the first or second specific antibody was replaced by a nonspecific antibody, and the sequence of staining was reversed.

Controls

The following controls were included for the specificity of the immune reaction: omission of the primary antibody and/or substituting the primary antibody with mouse serum or murine myeloma immunoglobulin G (IgG)-(MOPC21, Sigma); for the stainings with utr-1 and TNFα MAbs, the immunoreactivity was blocked with excess recombinant human TNFα.[8] Significant inhibition of staining was obtained at 1 μg/ml.

Paraffin Sections

Formalin-fixed paraffin-embedded material from utr-1–positive tissues was sectioned and immunostained as described above without or with previous pronase treatment (0.1%, 15 minutes at 37°C).

Reagents

An MAb against the p75 TNFR receptor protein, utr-1, was previously described.[7] The antibody htr-19 (M.

Brockhaus et al, unpublished data) was derived from a mouse immunized with p55 TNFR in an anologous way, as described for other anti-p55 TNFR antibodies.[8] Monoclonal antibodies against TNFα, tnf-14 were generated and characterized by standard procedures (M. Brockhaus, unpublished data). Monoclonal antibodies against resting and activated T and B lymphocytes (T11, T4, T8, L26, Tac) and dendritic reticulum cells and Langerhans cells (DRC1, T6) were obtained from Dakopatt (Glostrup, Denmark). Rabbit antibodies against TNFβ were purchased from Genzyme (Boston, MA) and Endogen (Bioreba, Basel, Switzerland).

Results

TNF Receptor Expression in Normal Lymphoid Tissues

Immunoreactivity of various monoclonal antibodies against TNFR from both the htr and utr series were tested. The best results were obtained with htr-19 and utr-1 on cryostat sections from frozen tissues, whereas no staining was obtained on paraffin sections. The immunoreactivity of both antibodies was confined to the lymphohistiocytic tissue, which includes the thymus and the secondary lymphoid organs such as spleen, tonsils, lymph nodes, mucosa, and associated lymphoid tissue.

Serial cryostat sections of tonsils were incubated with utr-1, htr-19, DRC1, Tac or T-cell MAb. Htr-19 immunoreactivity was confined to follicular DRC in germinal centers and not found at other sites (Figure 1C). The staining pattern with htr-19 was comparable to that obtained with DRC-1 antibody (not shown). Incubation with the utr-1 MAb resulted in a strong staining of interdigitating reticulum cells (IDC) and of activated T lymphocytes in the interfollicular T-cell area (Figure 1A). The distribution of utr-1 reactive cells overlapped with that of cells expressing the IL-2 receptor (Tac) in the T-cell area (Figure 1B). Based on sequential incubation with utr-1 and Tac on serial sections, it is concluded that utr-1 reactive lymphocytes and a subpopulation of IDCs coexpress the Tac protein. Finally the T6-positive Langerhans cells in the epithelium stained only with the utr-1 antibody (not shown). The specificity of the immunoreaction was supported by negative controls obtained by substitution or omission of the primary antibody, and by competition with excess TNFα for utr-1 staining.

A similar staining pattern was observed in lymph nodes (Table 1); little if any utr-1 reactive cells were found in germinal centers and the mantel zone of the follicle; the interfollicular T-cell area (paracortex) gave a distinct utr-1 staining of the IDCs and of T lymphocytes; in the medullary cord only little staining was evident. In the lymphoid tissues associated with the mucosae of the gut and the bronchus, a similar pattern of utr-1 reactive cells was found in T-cell areas.

In the thymus, only the medulla showed staining with the TNFR MAbs. Medullary utr-1 reactive cells comprised dendritic cells and lymphoblasts, which costained with Tac protein of the IL-2R (Figure 1E). No utr-1 reactivity was found in thymic epithelium nor in cortical thymocytes. There was a faint staining of dendritic cells with the htr-19 antibody. Hasall's bodies gave no reaction with either antibody.

Human bone marrow cells gave no immunoreactivity with either utr-1 or htr-19 antibody. On peripheral blood lymphocytes, a faint membrane staining was found with utr-1, but not with htr-19. With mitogenic activation, utr-1 reactivity increased (data not shown).

In the spleen (Figure 1F), utr-1 reactive cells comprising dendritic cells and lymphoblasts were located mainly in the T-cell area (periarterial lymphatic sheath); only a few utr-1 reactive cells were found in the mantle zone and follicles of the B-cell area.

In nonlymphoid tissues, the TNFR distribution also was investigated (Table 1). Scattered utr-1–positive, mononuclear cells in the interstitial space of kidneys, mucosae of various organs, and at inflammatory sites were observed. In inflammatory lesions, utr-1 reactive mononuclear cells from chronic infiltrates showed variable staining with utr-1 antibody. Endothelial cells and fibroblasts from various organs showed no detectable staining with either antibody.

TNFα Expression in Tissues

To obtain information on the spatial relationship of TNFR expression and TNF production sites, TNFα tissue expression was investigated using a MAb. TNFα reactive cells were confined preferentially to the lymph follicles (Figure 1D); within this structure the follicular DRCs with their cytoplasmic projections stained distinctly with anti-TNFα antibody, the immune reactivity was less in the mantle zone, and was almost absent in the interfollicular T-cell area of lymphoid organs. The staining with TNFα of DRCs was superimposable to that with htr-19 MAb. In the thymus, only little immunoreactivity was found with TNFα antibody, which was restricted to medullary dendritic cells. In the bone marrow, cells with monocytic morphology contained TNFα.

For the specificity of the immunoreactivity, incubations with TNFα antibody were performed in the presence of excess TNFα, which reduced the TNFα staining. Attempts to identify cells producing lymphotoxin (TNFβ) in the tissue with two different polyclonal antibodies failed.

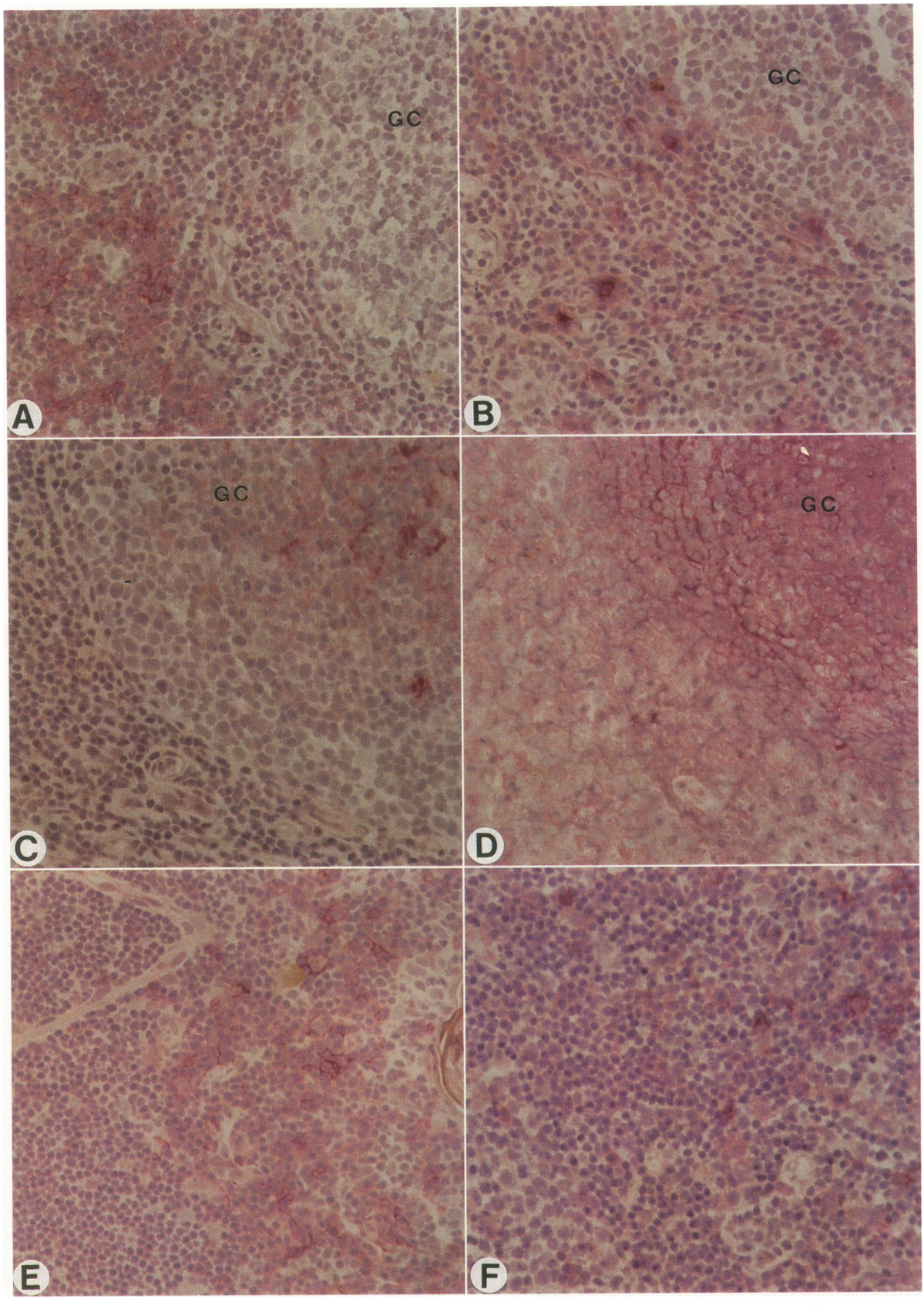

Figure 1. *Immunostaining for TNFα receptor on serial cryostat sections of human tonsils using utr-1 and hrt-19 MAbs* (**A–D**) *and on thymus and spleen (APAAP technique; magnification, ×320).* **A**: *utr–1–positive lymphocytes and IDC's in T-cell area.* **B**: *tac-positive cells in the same location.* **C**: *htr-19–positive DRCs in germinal centers (GC).* **D**: *Immunostaining with TNFα essentially of cells in the germinal center.* **E**: *Thymus: utr-1–positive cells in medulla.* **F**: *Spleen: utr-1–reactive cells in T-cell area.*

Table 1. *Distribution of TNFα Receptor Proteins in Normal Lymphoid Tissues*

Organ*	n	Area†	TNF-receptor protein		IL-2 receptor	
			utr-1	htr-19	Tac	TNFα
Tonsil	6	TA	+ + +	−	+ + +	+
		MZ	+	−	+	+
		GC	+	+	+	+ +
Lymph node	6	TA	+ + +	−	+ + +	(+)
		MZ	+	−	+	+
		GC	(+)	+	+	+ +
Spleen	3	TA	+ + +	−	+ +	−
		MZ	+	−	+ +	−
		GC	−	+	−	+
		RP	+	−	+	−
Thymus	2	Cortex	−	−	−	−
		Medulla	+ + +	+	+ +	−
Skin	2	Langerhans cells	+ +	−	+	−
Mucosa-associated lymphoid tissue	4	TA	+ + +	−	+ +	−
		MZ	+	−	+	−
		GC	(+)	+	(+)	+
Kidney	12	Reticulum cells (interstitial)	+ +	−	+	

* Organs examined that were negative included liver, heart, lungs, brain, adrenals, uterus, ovary, testes, prostate, stomach, and intestines.
† Immunoreactivity within various areas (TA, T-cell area; MZ, mantle zone or marginal zone for the spleen; GC, germinal center; RP, red pulp) was assessed by a semiquantitative score (0–3+).

TNFα Receptor Expression in Inflammation

The role of TNF and its receptor was investigated in reactive and inflammatory lymph nodes (Figure 2A–C). In hyperplastic lymph nodes, the number and intensity of utr-1 and htr-19 reactive cells were increased. Within the T-cell area, a distinct increase of utr-1–positive activated lymphocytes and dendritic cells could be observed. Epithelioid cells in granulomas and giant cells showed variable utr-1 positivity. Furthermore cells coexpressing Tac and utr-1 also were increased in the mantle zone and sometimes in the center of the follicle (Table 2). Concomitant with increased utr-1 expression, TNFα immunoreactivity also was found in epitheloid cells and giant cells and in hyperplastic lymph follicles.

TNFα Receptor Expression in Lymphoproliferative Disease

A series of Hodgkin and non-Hodgkin lymphomas was investigated for utr-1, htr-19, and TNFα immunoreactivity (Table 3). Selected cases are shown in Figure 2D–G). Among the non-Hodgkin lymphomas, most B- cell lymphomas expressed no or very little utr-1 reactivity, whereas T-cell lymphomas were more often utr-1 positive. A typical example of a non-Hodgkin, Ki-1–positive T-cell lymphoma is given in Figure 2E. Interestingly utr-1 reactivity was correlated to some extent with a high-grade malignant phenotype of lymphoma cells. Htr-19 reactivity was almost always absent. A single case of Hodgkin's lymphoma, however, gave a distinct reaction of Reed–Sternberg multinucleated giant cells with htr-19 (Figure 2F) and of Hodgkin cells with TNFα MAb (Figure 2G). Utr-1 reactivity often was associated with the expression of the Tac protein, but in some cases both receptors were expressed independently from each other. Because malignant lymphomas often are associated by a dense cellular infiltrate consisting of Tac- and utr-1–positive mononuclear cells, the distinction of the lymphoma cells from the reactive infiltrate cells sometimes was difficult.

Discussion

Immunohistochemistry using two specific monoclonal antibodies[8] revealed a unique distribution of the two

Table 2. *Expression of TNF Receptor Proteins in Lymphadenitis (LA)**

Diagnosis	Number of cases	utr-1				htr-19	
		TA	MZ	GC	EC	GC	EC
Nonspecific LA	4	+ + +	+	+	−	+	−
Acute LA with abscess	1	+ + +	+	−	−	+	−
Epitheloid cell LA	3	+ + +	+ +	+	+	+	−
Sarcoidosis	2	+ +	+	−	+	+	−

* Immunoreactivity in T-cell (TA) and B-cell areas (MZ, mantle zone; and GC, germinal center; EC, epitheloid cells) was assessed by semiquantitative score (0–3+).

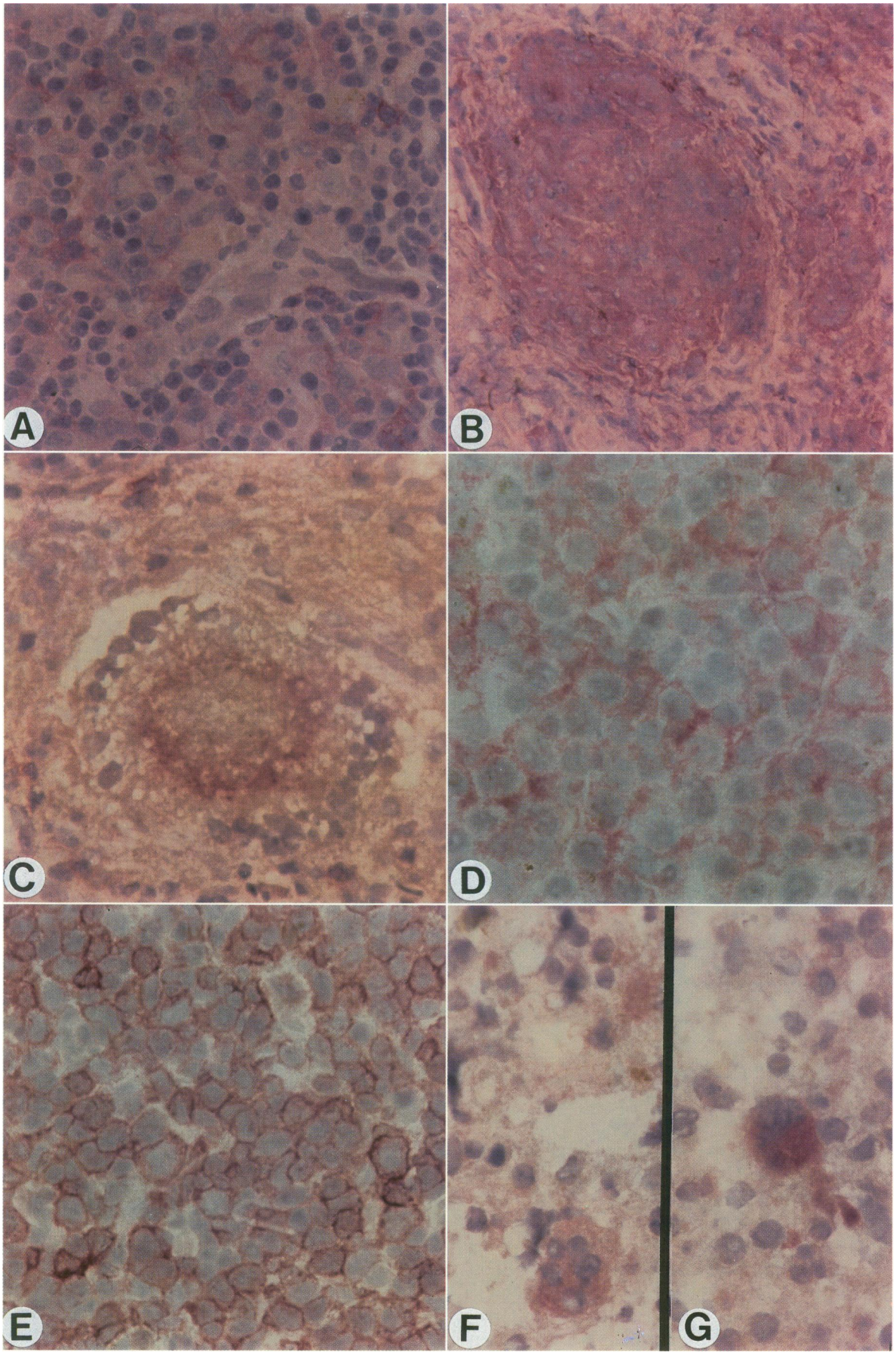
A
B
C
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Figure 2. *Immunostaining of cryostat sections from human lymph nodes with reactive and neoplastic changes.* **A**: *Acute lymphadenitis with increased utr-1–positive cells.* **B**: *Epithelioid cell granuloma stained with TNFα antibody.* **C**: *Sarcoidosis with utr-1–positive multinucleated giant cells.* **D**: *B non-Hodgkin lymphoma (utr-1).* **E**: *T non-Hodgkin lymphoma, Ki-1 positive, pleomorphic type (utr-1).* **F**: *Hodgkin lymphoma with Reed–Sternberg cell (htr-19).* **G**: *Hodgkin cell from the same case (TNFα).*

types of TNFRs in normal and pathologic human tissues. The p55 protein reacting with the htr-19 antibody constituted a minor population of TNFR expressed in DRCs of germinal centers. The p75 protein reactive with the utr-1 antibody represented a major population of TNFR-positive cells consisting of IDCs and activated lymphocytes in the interfollicular T-cell area. Based on sequential staining of serial sections and double staining, a high percentage of utr-1–positive lymphocytes and IDCs coexpress the Tac protein of the interleukin-2 receptor. The distribution pattern of both types of TNFRs in various secondary lymphoid tissues was similar.

TNFR distribution was compared with the immunostaining of the same tissues using antibody directed against TNFα and TNFβ. While the available anti-TNFβ antibodies showed no staining, the immunoreactivity of TNFα antibodies was strong and essentially located in the germinal center: DRCs with projections of the cell bodies into the mantle zone were stained, only a few dendritic cells stained in the T-cell area. These results are in agreement with earlier publications,[9,10] although the antibodies used in the present study were probably more sensitive.

Although the immunohistochemical method has its limitation, the present data suggest that the main source of TNFα is the macrophage-derived DRC, which also expresses the p55 TNFR protein. In the absence of a suitable antibody, the nature of TNFβ-producing cells cannot be determined in tissues. By contrast, the p75 TNFR sites are anatomically separated and are located mainly in the T-cell area.

Table 3. *Expression of TNF Receptor Proteins in Lymphoproliferative Disorders*

Diagnosis	TNF receptor utr-1	TNF receptor htr-19	Tac*
B-cell, non-Hodgkin's lymphoma			
Low grade			
Hairy cell	0/1	0/1	0/1
Follicular, centroblastic-cytic	0/3	0/3	1/3
Diffuse, centroblastic-cytic	0/4	0/4	1/4
High grade			
Centroblastic	3/9	0/9	4/9
Immunoblastic	3/3	0/3	2/3
Burkitt	0/1	0/1	0/1
T-cell, non-Hodgkin's lymphoma			
Low grade			
Mycosis fungoides	0/1	0/1	0/1
Lennert	0/2	0/2	0/2
Angioimmunoblastic	2/2	0/2	2/4
High grade			
Immunoblastic	2/2	0/2	2/4
Pleomorphic Ki-1 +	2/2	0/2	2/4
Hodgkin's lymphoma	2/4	1/4	3/4

* Immune reactivity of tumor cells.

Both DRCs of germinal centers and IDCs of the T-cell area are known to function as antigen-presenting cells and are apparently responsive to the same lymphokine. It appears that the cooperation with the respective antigen-reactive lymphocyte requires a different receptor structure that is recognized by the utr-1 and htr-19 MAb. The fact that activated lymphocytes coexpress receptors for IL-2 and TNF is in accordance with previous findings of p75 TNFR expression on activated T cells[20] and supports *in vitro* observations that TNFα plays a role in the activation of lymphocytes.[11–13,21]

These results demonstrate, for the first time, a distinct distribution of the two TNFR proteins in the lymphoid tissue. In view of the differential location of cells producing and responding to this cytokine, autocrine and paracrine signaling pathways of TNFα are suggested that use different receptor structures. No information is presently available to determine if receptor proteins also have differing functions.

Investigations on pathologic tissues might promote our understanding of the role of TNFα in disease. In reactive lymph nodes, the number of p75 (utr-1)-positive cells was increased in the T-cell area; epitheloid cell granulomas and giant cells in sarcoidosis expressed p75 TNFR, suggesting a role for TNF in their formation.[22] Experimental evidence for the role of TNF in cancer is a matter of debate. While some experimental tumors are highly sensitive to TNF-induced hemorrhagic necrosis, most human cancers are resistant.[23] An *in vitro* comparison of human tumors of different origin showed that T- or B-cell malignancies were resistant, whereas cell lines derived from epithelial cancer are responsive to a cytotoxic effect of TNF. Among epithelial cancer, established mammary cancer lines were inhibited by TNF, whereas normal breast epithelium was not affected by TNF.[24,25] Scheurich et al[26] investigated the possible correlation of TNF responsiveness with receptor expression on different cell lines. TNFR expression on individual tumor cell lines did not correlate with a TNF-sensitive phenotype.

A recent investigation on Hodgkin's disease-derived cell lines showed that the Hodgkin cells produced both

TNFα and TNFβ (lymphotoxin[27]). In our investigation, a limited number of Hodgkin and non-Hodgkin lymphomas were analyzed for TNFR expression. The p75 TNFR was found mainly on lymphoma cells with a high-grade malignant phenotype in both T and B non-Hodgkin lymphomas. The p55 (htr-19) antigen was not expressed on our material, except for a single case of Hodgkin's lymphoma. TNFα expression was not tested in a systematic manner on these lymphoma sections. Our investigation showed that p75 TNFR (utr-1) and the IL-2R (Tac) protein are not necessarily associated on single cells. These preliminary data suggest that further studies on lymphomas might establish TNFR expression as a prognostic marker.

Surprisingly the present investigation with both TNF receptor antibodies did not yield any staining of endothelial cells, smooth muscle cells, and fibroblasts, cells that are known to respond to TNF.[15,16,28,29] Evidence for an important role of TNF and other cytokines in normal and pathologic vascular endothelial responses was recently reviewed.[30] The absence of immunoreactivity may be due to a rather low number of receptor molecules expressed, low sensitivity of the immunohistochemical method, or fundamental difference between cultured cells, cell lines, and the *in vivo* situation. The only TNFR-positive cells in non-lymphoid tissues found were dendritic reticulum cells, which are located in the intersitial space of various organs such as kidneys, lung, and mucosa, expressed variable amounts of the p75 protein.

In summary, an unique and very restricted distribution of both TNF receptor proteins is reported. Receptor expression was confined to the lymphoreticular tissue. The p75 protein was found mainly in the T-cell area, whereas the p55 protein was restricted to DRCs of germinal centers. The latter coincides with the site of TNFα production. These results suggest the existence of two TNF receptor systems, which differ in their signaling through autocrine or paracrine pathways.

References

1. Beutler B, Cerami A: The biology of cachectin/TNF—A primary mediator of the host response. Ann Rev Immunol 1989, 7:625–655
2. Paul NL, Ruddle NH: Lymphotoxin. Ann Rev Immunol 1988, 6:407–438
3. Pennica D, Nedwin GE, Hayflick JS, Seeburg PH, Derynck R, Palladino MA, Kohr WJ, Aggarwal BB, Goeddel DV: Human tumor necrosis factor: Precursor, structure, expression and homology to lymphotoxin. Nature 1984, 312:724–729
4. Old LJ: Tumor necrosis factor (TNF). Science (Washington, DC) 1985, 230:630–634
5. Sung SJ, Bjorndahl JM, Wong CY, Kato HT, Fu SM: Production of tumor necrosis factor/cachectin by human T cell lines and peripheral blood T lymphocytes stimulated by phorbol myristate acetate and anti-CD3 antibody. J Exp Med 1988, 167:937–944
6. Amino N, Linn ES, Pysher TJ, Mier R, Moore GE, deGroot, LJ: Human lymphotoxin obtained from established lymphoid lines: Purification characteristics and inhibition by anti-immunoglobulin. J Immunol 1974, 113:1334–1345
7. Hohmann HP, Remy R, Brockhaus M, van Loon APGM: Two different cell types have different major receptors for human tumor necrosis factor (TNFα). J Biol Chem 1989, 264:14927–14934
8. Brockhaus M, Schoenfeld HJ, Schlaeger EJ, Hunziker W, Lesslauer W, Loetscher H: Identification of two types of tumor necrosis factor receptors on human cell lines by monoclonal antibodies. Proc Natl Acad Sci USA 1990, 87:3127–3131
9. McCall JL, Kankatsu Y, Shinsaku F, Parry BR: In vivo immunohistochemical identification of tumor necrosis factor/cachectin in human lymphoid tissue. Am J Pathol 1989, 135:421–425
10. Ruco LP, Stoppacciaro A, Donatella P, Boraschi D, Santoni A, Tagliabue A, Ucchini S, Baroni CD: Immunoreactivity for IL-1 beta and TNF alpha in human lymphoid and nonlymphoid tissues. Am J Pathol 1989, 135:889–897
11. Ranges GE, Bombara MP, Ramani AA, Rice GG, Palladino MA: Tumor necrosis factor-α as a proliferative signal for an IL-2 dependent T cell line: Strict species specificity of action. J Immunol 1989, 142:1203–1208
12. Scheurich P, Thomas B, Ucer U, Pfizenmaier K: Immunoregulatory activity of recombinant human tumor necrosis factor (TNF-α): Induction of TNF receptors on human T cells and TNF-α mediated enhancement of T cell responses. J Immunol 1987, 138:1786
13. Kehrl J, Miller A, Fauci A: Effect of tumor necrosis factor α on mitogen activated human B cells. J Exp Med 1987, 166:786
14. Imamura K, Spriggs D, Kufe D: Expression of tumor necrosis factor receptors on human monocytes and internalization of receptor bound ligand. J Immunol 1987, 139:2989–2992
15. Lin JX, Vilcek J: Tumor necrosis factor and interleukin-1 cause a rapid and transient stimulation of c-fos and c-myc mRNA levels in human fibroblasts. J Biol Chem 1987, 262:11908–11911
16. Collins T, Lapierre LA, Fiers W, Strominger LJ, Pober JD: Recombinant human tumor necrosis factor increases mRNA levels and surface expression of HLA-A,B antigens in vascular endothelial cells and dermal fibroblasts in vitro. Proc Natl Acad Sci USA 1986, 83:446–450
17. Smith CA, Davis T, Anderson D, Solam L, Beckmann MP, Jerzi R, Dower SK, Cosman D, Goodwin RG: A receptor for tumor necrosis factor defines an unusual family of cellular and viral proteins. Science 1990, 248:1019–1023
18. Loetscher H, Pan YCE, Lahm HW, Gentz R, Brockhaus M, Tabuchi H, Lesslauer W: Molecular cloning and expression of the human 55 kd tumor necrosis factor receptor. Cell 1990, 61:351–359
19. Schall TJ, Lewis M, Koller KJ, Lee A, Rice GC, Wong GHE, Gatanaga T, Granger GA, Lentz R, Raab H, Kohr WJ, Goeddel DV: Molecular cloning and expression of a receptor for human tumor necrosis factor. Cell 1990, 61:361–370

20. Dembic Z, Loetscher H, Gubler U, Pan YCE, Lahm HW, Gentz R, Brockhaus M, Lesslauer W: Two human TNF receptors have similar extracellular, but distinct intracellular, domain sequences. Cytokine 1990, 2:231–237
21. Kuhweide R, Van Damme J, Ceuppens JL: Tumor necrosis factor-α and interleukin 6 synergistically induce T cell growth. Eur J Immunol 1990, 20:1019–1025
22. Chensue SW, Otterness IG, Higashi GI, Forsch CS, Kunkel SL: Monokine production by hypersensitivity (Schistosoma mansoni EGG) and foreign body (sephadex bead)-type granuloma macrophages. J Immunol 1989, 142:1281–1286
23. Old LJ: Tumor necrosis factor. *In* Bonavida B, Granger G, eds. Tumor Necrosis Factor: Structure, Mechanism of Action, Role in Disease and Therapy. Basel, Karger, 1990, pp 1–30
24. Williamson BD, Carswell EA, Rubin BY, Prendergart JS, Old LJ: Human tumor necrosis factor produced by human B cell lines. Synergistic cytotoxic interaction with human interferon. Proc Natl Acad Sci USA 1983, 80:5397–5401
25. Dollbaum C, Creasey AA, Dairkee SH, Hiller AJ, Rudolf AR, Lin L, Vitt CH, Smith HS: Specificity of tumor necrosis factor toxicity for human mammary carcinomas relative to normal mammary epithelium and correlation with response to doxorubicin. Proc Natl Acad Sci USA 1988, 85:4740–4744
26. Scheurich P, Uecer U, Krönke M, Pfizenmaier K: Quantification and characterization of high-affinity membrane receptors for tumor necrosis factor on human leukemic cell lines. Int J Cancer 1986, 38:127–133
27. Kretschmer C, Jones DB, Morrison K, Schlüter C, Feist W, Ulmer AJ, Arnoldi J, Matthes J, Diamantstein T, Flad HD, Gerdes J: Tumor necrosis factor α and lymphotoxin production in Hodgkin's disease. Am J Pathol 1990, 137:341–351
28. Stolpen AH, Guinan EC, Fiers W, Pober JS: Recombinant tumor necrosis factor and immune interferon act singly and in combination to reorganize human vascular endothelial cell monolayers. Am J Pathol 1986, 123:16–24
29. Warner SJC, Libby P: Human vascular smooth muscle cells. Target for and source of tumor necrosis factor. J Immunol 1989, 142:100–109
30. Pober JS: Cytokine-mediated activation of vascular endothelium. Am J Pathol 1988, 133:426–433