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TNF staining of graft biopsy in renal transplantation

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Tumour Necrosis Factor (TNF) is a cytokine which may be found in patients' plasma and urine in association with acute rejection in renal transplantation [1]. TNF is produced mainly by macrophage/monocytes and activated lymphocytes and its release in acute rejection may damage the nephron leading to renal dysfunction. However localization of TNF in renal grafts has not yet been demonstrated. We investigated TNF localization in renal graft tissue and the association with acute rejection compared with non-immunological events (cyclosporine toxicity and acute tubular necrosis) in graft biopsy.

Key words: Tumor necrosis factor – Renal transplantation – Graft biopsy

Materials and methods

We used 50 graft biopsy specimens from 44 renal transplant patients in this study.

TNF staining (immunoperoxidase method: ABC-HRP)
1. Deparaffinise with xylene 5 mins.
Absolute ethanol 2 changes, 3 mins each.
95% ethanol 2 changes, 3 min each.
Rinse in distilled water.
Wash in PBS.
2. Trypsinise with 1% trypsin for 20 mins.
Wash in PBS. Tap off.
3. Block with 10% non-immune rabbit serum for 10 min.
4. Incubate with mouse primary antihuman TNFα monoclonal antibody (J1D9) 60 min at 37 °C
Wash with PBS.
5. Incubate with biotinylated second antibody (anti-mouse) 5 min at room temperature.
Wash with PBS.
6. Incubate with enzyme conjugate 5 min at room temperature.
Wash with PBS.
7. Incubate with substrate chromogen mixture 5 min at room temperature.
Wash with distilled water.
8. Counterstain with haematoxylin 3 min at room temperature.
Wash well with tap water.
9. Mount section using dehydrate + mount with DPX.

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Results

Localization of TNF staining in the nephron

Staining was observed mainly in cells of the distal tubules and Henle's loop. The staining pattern was cytoplasmic or luminal in them and occasionally patchy in proximal tubular cells less strongly. Occasionally, lymphoid cells (mainly small lymphocytes but some macrophages) were stained. No staining was observed in the vascular endothelium or the glomerular mesangial cells (Table 1, Figs. 1 and 2).

Association with graft status

No staining was observed in the donor kidneys before transplantation. TNF was observed mainly in the distal tubules in acute rejection episodes. During chronic vascular rejection TNF was found in half of the biopsies, whereas in primary non-function or cyclosporine toxicity, TNF was not a usual feature (Table 2).

Discussion

TNF was observed mainly in distal tubular cells and Henle's loop in graft biopsy specimens of acute rejection. This characteristic staining pattern reflects that Tamm-

Table 1. Localization of TNF in renal graft biopsies

Glomerulus	Proximal tubular cells	Distal tubular cells Henle's loop	Vascular endothelium
–	±	+	–

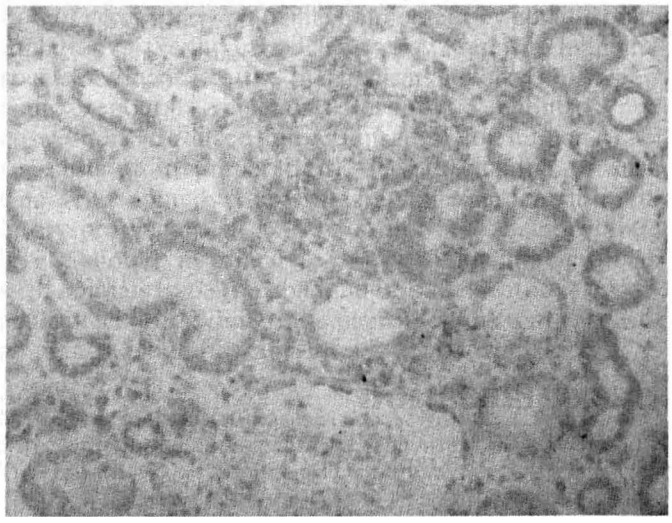

Fig. 1. Acute rejection. TNF was stained mainly in destal tubules. Proximal tubules were stained less strongly and patchy. Some infiltrated lymphoid cells were stained. No staining was observed in glomerulus

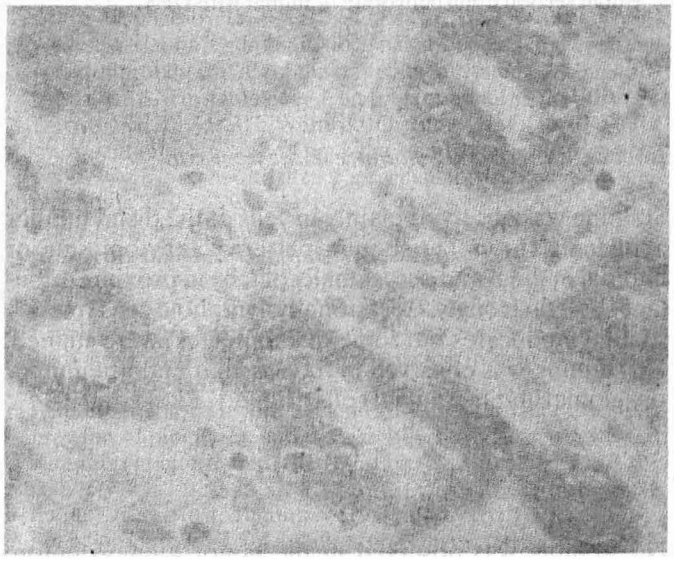

Fig. 2. Acute rejection. The staining pattern of distal tubular cells was cytoplasmic. Some infiltrated lymphoid cells were stained.

Horsfall glycoprotein (THG) which tends to ligand with TNF, is localized particularly in the distal tubular cells and collecting ducts. TNF may be produced by activated macrophage/monocyte and lymphocytes in acute rejection and in the case of severe acute rejection, some infiltrating cells were strongly stained [2]. Staining of TNF might indicate the severity of acute rejection and the active phase of the rejection process even in chronic vascular rejection.

TNF staining is never observed in donor kidney biopsies before grafting and rarely observed in non-immunological events (cyclosporine toxicity or acute tubular necrosis in primary non-functioning graft). TNF staining might be of value in differentiating between these non-immunological events and active rejection.

Table 2. TNF staining in various graft status

Donor's kidney	AR	CVR	Primary non-function	CyA Toxicity
0/5 (0%)	17/23 (74%)	7/14 (50%)	2/6 (33%)	0/2 (0%)

AR, Acute rejection; CVR, chronic vascular rejection; CyA, cyclosporine

Summary

TNF staining was not observed in the glomerulus, the vascular endothelium, and the distal tubules stained better than proxymal tubles. TNF staining occurred more frequently in acute rejection compaired with non-immunological events.

Conclusion

The pattern of TNF staining in renal graft biopsy may be of value in the understanding of the action of TNF in acute rejection and may differentiate between active rejection and non-immunological events.

References

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