Monitoring the acute phase response: comparison of tumour necrosis factor (cachectin) and C-reactive protein responses in inflammatory and infectious diseases

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SUMMARY   The relation between the inflammatory cytokine tumour necrosis factor-α (TNF or cachectin), which induces acute phase responses, and an established acute phase protein, C-reactive protein, was studied in various infectious and inflammatory diseases in man. All cases with very high serum concentrations of C-reactive protein (150 to 400 mg/l; normal reference value < 10 mg/l) also had raised serum concentrations of TNF (53 to 705 ng/l; normal reference value < 40 ng/l). In 19 out of 91 (21%) of the cases, however, a raised TNF concentration without correspondingly raised C-reactive protein concentration was also noted. Conversely, in 23 out of 106 (22%) cases raised C-reactive protein was observed in the absence of a raised TNF concentration. The ratios were high in allograft rejection and low in myocardial infarction and Kawasaki's disease. The highest mean concentration of circulating TNF was found in bacterial infections, graft rejection, and myocardial infarction.

It is concluded that although high C-reactive protein concentrations are usually accompanied by raised TNF concentrations, there are pronounced relative variations in the serum concentrations of these proteins in various disease states, suggesting that there may be independent, disease specific regulatory pathways for TNF and C-reactive protein.

Tumour necrosis factor-α (TNF or cachectin) is a hormone-like intercellular signal peptide with a role in host defence processes against tumour cell growth and parasite infection. Recent studies have also implicated TNF in the regulation of normal cell metabolism and as an effector molecule in various processes related to inflammation.[1 2] TNF is elaborated mainly by cells of the mononuclear phagocyte system and induces several phenomena associated with the acute phase response such as fever, granulocyte activation, and hepatic protein synthesis. Raised concentrations of circulating TNF in certain inflammatory diseases have recently been reported.[3-11] To gain insight into the association between circulating TNF and the acute phase response in disease, TNF was measured in various pathological conditions and compared with the concentration of C-reactive protein, and in some instances, also with serum amyloid A protein (SAA), acute phase proteins known to be produced by the liver.

Patients and methods

Cross sectional studies were carried out in 139 patients with rheumatoid arthritis (n = 22), systemic lupus erythematosus (n = 17), Kawasaki's disease (n = 39), myocardial infarction (n = 10), renal allograft rejection (n = 9), human immunodeficiency virus (HIV) infection (n = 21) or systemic bacterial diseases (n = 21). Serum samples from 40 healthy blood donors served as controls.

Longitudinal studies were carried out in cases of myocardial infarction (11, 15, and 19 samples, respectively/patient), renal allograft rejection (23 and 24 samples, respectively/patient), Gram negative septicaemia (12 samples/patient), AIDS (six samples/patient), Crohn's disease (eight samples/patient) and histiocytosis (11 samples/patient).

TNF in serum was measured using a sensitive double antibody radioimmunoassay.[1 2] The detection limit of the assay is 7 ng/l. To calculate the intra-assay and interassay coefficients of variation sera were analysed over 26 days in three parallel determinations

Table 1 *Correlation between serum TNF and C-reactive protein concentrations in disease expressed as means (SEM)*

Patient group/disease	(n =)	TNF (ng/l)	C-reactive protein (mg/l)	TNF v CRP r	TNF:C-reactive protein relative ratio
Rheumatoid arthritis	22	104 (25)	30 (7)	−0·13	3·5
Systemic lupus erythematosus	17	26 (27)	7 (3)	−0·03	3·6
Kawasaki's disease	39	34 (3)	47 (6)	0·28	0·7
Myocardial infarction	10	129 (32)	88 (23)	0·61	1·5
Renal allograft rejection	9	159 (34)	17 (6)	0·68	9·4
Systemic bacterial infections	21	327 (93)	94 (18)	0·30	3·5
HIV infection	21	74 (16)	17 (6)	0·53	4·2

in nine separate assays for the interassay assessment, and in 10 to 15 parallel measurements, each in one run, for intra-assay precision. The intra-assay and inter-assay coefficients of variation were 8·2% and 9·4%, respectively, for a TNF concentration of 15 ng/l, and 6·3% and 7·0%, respectively, for a concentration of 92 ng/l. The concentrations of TNF in healthy subjects (40 blood donors) was less than 40 ng/l.

C-reactive protein was measured by radial immunodiffusion using specific antiserum (Orion Diagnostica, Finland) and C-reactive protein reference serum (Behringwerke AG, West Germany) as standard. The C-reactive protein concentration in healthy subjects (40 blood donors) was less than 10 mg/l. SAA was measured by radial immuno-diffusion as described before.[13] Normal reference values of SAA are < 15 mg/l.[14]

Results were expressed as mean ± standard error of mean, and as ranges. Correlation coefficients (r) were calculated using linear regression analysis.

Results

Serum TNF concentrations were highest in bacterial infections (327 (93) ng/l, renal allograft rejection (159 (34) ng/l), and myocardial infarction (129 (32) ng/l). The concentrations of TNF in rheumatoid arthritis (104 (25) ng/l) and in HIV infection (74 (16) ng/l) were moderately raised; the mean TNF concentrations in systemic lupus erythematosus were within the normal reference range (table 1). A tendency towards a

positive correlation between TNF and C-reactive protein concentrations was seen in both cross sectional (table 1) and longitudinal (table 2) studies of patients with renal allograft rejection (fig 1), myocardial infarction (figs 2 and 3) and in infections: no such correlation was found in rheumatic diseases (table 1). The TNF:CRP ratios were low in myocardial infarction and Kawasaki's disease and high in renal allograft rejection (table 1). Normal TNF concentrations were seen in most cases accompanied by normal C-reactive protein concentrations. In 23 of 106 (22%) cases, however, raised C-reactive protein concentration (> 10 mg/l) was found in the absence of raised TNF. Conversely, in 19 of 91 (21%) cases a raised TNF concentration was noted, in spite of a normal C-reactive protein concentration. In all cases with a very high C-reactive protein concentration (range 150 to 400 mg/l) a raised TNF concentration was also found (range 53 to 705 ng/l).

Table 2 *Correlation between serum TNF and C-reactive protein concentrations in individual patients during follow up*

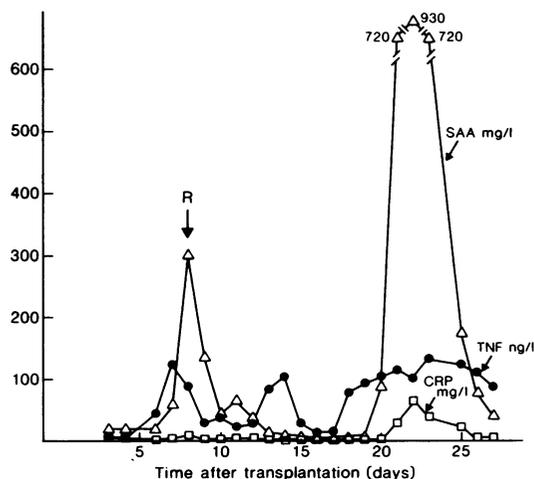| Disease	Number of samples		TNF v C-reactive protein r
Myocardial infarction	19	(case 1)	0·31
	15	(case 2)	0·63
	11	(case 3)	0·75
Renal allograft rejection	24	(case 1)	0·48
	23	(case 2)	0·45
Septicaemia	12		0·59
Crohn's disease	8		−0·13
Histiocytosis	11		−0·23
AIDS	6		0·33

Fig 1 *Correlation between TNF (●–●), C-reactive protein (□–□), and SAA (△–△) responses in a 30 year old man following cadaveric renal transplantation and immunosuppressive treatment with azathioprine and cyclosporine. The acute phase reaction peaking on the 22nd day after transplantation was associated with a fever episode of unknown aetiology. R indicates a graft rejection episode.*
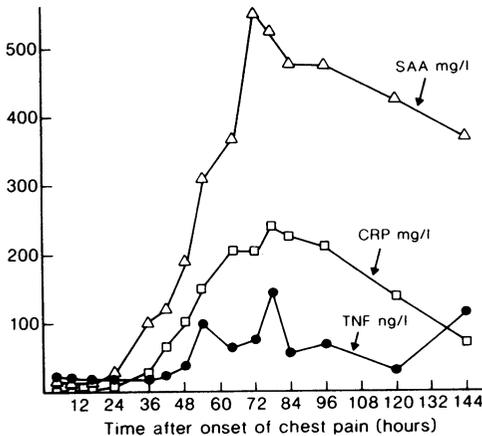
Fig 2 *Correlation between serum TNF (●–●), C-reactive protein (□–□), and SAA (△–△) in a 66 year old man who had had an acute, large, inferoapical transmural myocardial infarction. He died seven days after the onset of chest pain.*

Discussion

TNF is an inflammatory cytokine implicated in the pathogenesis of several conditions including septic shock,[4][15] cachexia,[16][17] graft rejection,[5] graft versus host disease,[18] cerebral malaria,[19] vasculitis in Kawasaki's disease[20] and haematological abnormalities in inflammatory diseases.[21] Raised concentrations of circulating TNF have recently been reported in various infectious, inflammatory, and malignant diseases.[3-12] Most of these represent states also characterised by an acute phase reaction and increased C-reactive protein concentrations. As TNF is known to induce acute phase protein synthesis in the liver,[22][23] the question arises whether a raised TNF concentration has the same clinical implications as a raised C-reactive protein concentration and what the association between the concentrations of these proteins in the circulation in various diseases is.

Analysis of the data showed that, irrespective of the underlying disease, very high C-reactive protein concentrations (≥ 150 mg/l) were always associated with increased circulating concentrations of TNF. There were, however, noticeable disease dependent variations of TNF concentrations and the correlation between high C-reactive protein concentrations and corresponding TNF concentrations was weak. This finding was also reflected in the pronounced variation in the relative TNF C-reactive protein ratios in disease. The ratio was high in immunological tissue injury (renal allograft rejection)—high TNF and low C-reactive protein responses. In contrast, tissue injury in association with myocardial infarction was characterised by low TNF: C-reactive protein ratios.

In 21% of the cases raised TNF concentrations was observed in the absence of an increased C-reactive protein concentration; most of these cases were associated with immunological tissue injury. Conversely, in 22% a raised C-reactive protein concentration was found in the absence of raised TNF; these occurred in different diseases including bacterial infections and rheumatoid arthritis.

Our results show that the TNF response in inflammation is disease specific and differs in several respects from the C-reactive protein response. Activation of monocytes and macrophages,[1][2] and possibly other cells as well,[24] is the primary event which initiates TNF production. Experimental studies show that a wide range of infectious and non-infectious agents can activate mononuclear phagocytes. Interestingly, the gene coding for TNF has been mapped to chromosome 6 in close linkage to the major histocompatibility genes.[25] The finding of abnormal TNF expression in conditions characterised by dysfunction of the regulation of the immune system is not, therefore, surprising.

TNF induces the synthesis of hepatic acute phase proteins by modulating gene expression during the acute phase response. TNF, however, is not the only humoral factor involved. Interleukin 1[26] and interleukin 6[23][27][28] also induce acute phase protein synthesis. The relative roles and possible interaction of the various cytokines in inducing C-reactive protein synthesis in man is not known, but the pronounced differences in C-reactive protein responses at certain
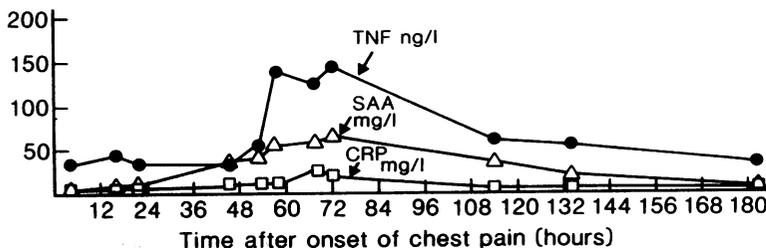
Fig 3 *Correlation between serum TNF (●–●), C-reactive protein (□–□), and SAA (△–△) in an 84 year old man with a non fatal anteriolateral myocardial infarction. Note the lesser acute phase protein response in this patient than in the patient shown in fig 2, although both patients had similar TNF response curves.*

TNF concentrations suggest that mediators other than TNF may also be implicated.

The different TNF:CRP ratios resulting from various acute inflammatory stimuli may also be explained by differences in consumption and clearance from the circulation. Anti-inflammatory and immunosuppressive drugs may also affect the metabolism of TNF and C-reactive protein differently. Using a bioassay for serum TNF, Waage *et al* have calculated that the half-life of TNF is about 70 minutes in patients with meningococcal septic shock.[29] In cancer patients receiving intravenous recombinant TNF, the half-life in blood was estimated at 20 minutes.[30] Data from studies on mice[31] and rats[32] show a great variation of the measured half-lives of SAA, depending probably both on experimental design and species difference. The in vivo half-life of labelled C-reactive protein in the circulation of rabbits has been reported to be four to six hours.[33] In man serial measurements of the serum concentrations of SAA and C-reactive protein following tissue injury[34] and ethiocholanolone administration,[35] however, show that the kinetics of these proteins are similar. Our results show that the kinetics of the TNF and SAA and C-reactive protein responses following tissue injury are also rather similar, though the correlation between TNF and C-reactive protein is considerably weaker than the correlation between C-reactive protein and SAA under these conditions.

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