Relationship of serum levels of interleukin-6, soluble interleukin-6 receptor and tumour necrosis factor receptors to the acute-phase protein response in advanced pancreatic cancer

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ABSTRACT

The level of the acute-phase response is a major predictor of survival in patients with advanced pancreatic cancer. This study examines the association between the acute-phase protein response, as determined by serum C-reactive protein, and serum levels of interleukin-6, soluble interleukin-6 receptor and the soluble tumour necrosis factor receptors in patients with pancreatic cancer. Thirty-four blood samples were collected from 13 patients with advanced pancreatic cancer. Samples were also collected from six healthy subjects. Levels of C-reactive protein, interleukin-6, soluble interleukin-6 receptor and soluble tumour necrosis factor receptors 55 and 75 were measured by indirect ELISA. Serum levels of C-reactive protein, interleukin-6 and soluble tumour necrosis factor receptors 55 and 75 were significantly higher in cancer patients than in controls. Levels of serum soluble interleukin-6 receptor were not significantly different between the two groups. In cancer patients, a significant positive association was found between the level of the acute-phase protein response and serum levels of interleukin-6, soluble tumour necrosis factor receptor 55 and soluble tumour necrosis factor receptor 75. No association was found between levels of soluble interleukin-6 receptor and any other factor. There is no significant relationship between the level of soluble interleukin-6 receptor and the acute-phase protein response in vivo and the biological role of soluble interleukin-6 receptor in the chronic inflammatory component of cachexia remains unclear.

INTRODUCTION

Pancreatic adenocarcinoma is the fifth most common cause of death from cancer in the Western World [1]. Despite advances in diagnosis and staging the median survival remains dismal at 4.1 months [2]. About 85% of patients with pancreatic cancer exhibit weight loss at diagnosis and this progresses inexorably until death [3]. It would appear that in many cancer patients the primary cause of death is this cachexia [4].

We have previously shown that the weight loss in patients with advanced pancreatic cancer is associated with the hepatic acute-phase protein response (APPR) [5]. The APPR involves a reprioritization of protein...
synthesis within the liver in response to trauma and inflammation. The roles of the APPR include limitation of tissue damage, isolation and destruction of infective organisms and the promotion of repair [6]. However, the presence of an APPR as measured by an elevated serum C-reactive protein (CRP) level is strongly associated with a shorter survival in patients with advanced pancreatic cancer [7]. Thus, in patients with cancer cachexia, the reprioritization of protein synthesis associated with the APPR may lead to persisting muscle catabolism and wasting which is detrimental in the long term.

Interleukin-6 (IL-6) appears to have a major role in inducing the APPR [8] but previously we have not been able to show a relationship between serum IL-6 levels and the APPR in patients with pancreatic cancer as measured by an elevated level of CRP [5]. Tumour necrosis factor (TNF) will also produce many of the features of cachexia when administered to humans [9] but significant serum levels are rarely detected in cancer [5]. However, elevated production of IL-6 and TNF by peripheral blood mononuclear cells isolated from patients with an APPR suggests that local production of these cytokines may be more important to regulation of the APPR than serum levels [5].

Of additional interest in the context of the APPR and cachexia are the receptors for TNF and IL-6. Two receptors for TNF of 55 and 75 kDa have been described [10]. Soluble forms of these receptors – sTNF-R55 and sTNF-R75 – appear to be shed from cells in response to TNF release, possibly to limit the activity of this cytokine [11]. TNF receptor levels are associated with disease severity in a number of inflammatory conditions [12–14]. In contrast to many other soluble receptors, it has been shown that the IL-6 signal may be delivered via binding to soluble IL-6 receptor (sIL-6R) and subsequent interaction of this complex with gp130 on the cell surface whether or not membrane-bound IL-6R is present [15]. The capacity of the cell to respond to IL-6 may depend not only on cell surface IL-6R and gp130 expression but also the level of sIL-6R, at least in vitro [16–18]. Serum levels of sIL-6R may therefore be a more important determinant of the response to IL-6 than serum levels of IL-6.

The aim of this study was to assess the relationship between serum levels of IL-6, sIL-6R, sTNF-R55 and sTNF-R75 and the APPR in patients with advanced pancreatic cancer.

PATIENTS AND METHODS

Subjects

After local ethics committee approval 13 patients gave written, informed consent for the collection of a total of 34 venous blood samples. All had an unequivocal diagnosis of unresectable pancreatic cancer based on histological or operative findings. All samples were taken at least 14 days after surgery or biliary drainage. No patient had clinical evidence of current infection. All repeated samples were taken at least 8 days apart. Samples were also obtained from six control subjects with no active medical conditions after gaining consent. Serum was stored at −70 °C until batch analysis by ELISA.

Cytokine, receptor and CRP ELISAs

IL-6, sIL-6R, sTNF-R55, sTNF-R75 and CRP were all measured by indirect ELISA. IL-6 was measured using a monoclonal anti-human-IL-6 antibody and peroxidase-conjugated Fab fragments of a murine monoclonal anti-human-IL-6 antibody (CLB, Amsterdam, Netherlands). The lower limit of detection was 0.25 pg/ml. sIL-6R was measured using a monoclonal anti-human-sIL-6R antibody and peroxidase-conjugated Fab fragments of a murine monoclonal anti-human-sIL-6R antibody (CLB, Amsterdam, Netherlands). The lower limit of detection was 4 ng/ml. sTNF-R55 and sTNF-R75 were measured using polyclonal and monoclonal anti-sTNF-R55 and anti-sTNF-R75 antibodies kindly provided by Dr W. A. Buurman, University of Maastricht, Netherlands [19]. The lower limit of detection was 190 pg/ml for sTNF-R55 and 2 ng/ml for sTNF-R75. CRP was measured using rabbit anti-human-CRP antibody and peroxidase-conjugated rabbit anti-human-CRP antibody (DAKO, High Wycombe, U.K.). The lower limit of detection was 1 mg/l. In all cases a standard curve was constructed from standards provided by the relevant suppliers.

Statistical analysis

Statistical analysis was carried out using Statview (Abacus Concepts Inc., Berkeley, CA, U.S.A.). Data are presented as medians (interquartile range) unless otherwise stated. Comparisons between control and patient groups were made using the Mann–Whitney U-test. Correlation was assessed using Spearman’s rank correlation coefficient. Results were considered to be statistically significant with a P value < 0.05.

RESULTS

Patients had a median age of 67 (51–76) years. Control subjects had a median age of 54 (50–62) years (P = 0.043 compared with patients). Seven patients had stage II disease, one had stage III and five had stage IV (Union Internationale Centre le Cancre system). The data for CRP, IL-6, sTNF-R55, sTNF-R75 and sIL-6R are summarized in Figure 1.

Patients had significantly higher serum levels of CRP [control: < 1 mg/ml; patients: 5.4 (2.6–22.2) mg/l, P = 0.001], IL-6 [0.5 (< 0.25–1.0) pg/ml versus 5.2...
Figure 1  Serum levels of interleukin-6 (IL-6), soluble tumour necrosis factor 55 (sTNF-R55) and 75 (sTNF-R75) receptors, C-reactive protein (CRP) and soluble interleukin-6 receptor (sIL-6R) in patients with pancreatic cancer (○) and control subjects (●). Medians are shown by a horizontal line. Comparisons were made by Mann–Whitney’s U-test.

Figure 2  Correlation between serum CRP and levels of (upper left panel) IL-6, (upper right panel) sTNF-R55, (lower left panel) sTNF-R75 and (lower right panel) sIL-6R in patients with pancreatic cancer
(Upper left panel) \( n = 34, \rho = 0.62, P = 0.0004 \); (upper right panel) \( n = 34, \rho = 0.59, P = 0.0008 \); (lower left panel) \( n = 34, \rho = 0.53, P = 0.0024 \); (lower right panel) \( n = 32, \rho = 0.17, P = 0.34 \) (not significant).
DISCUSSION

In this study we have demonstrated that patients with advanced pancreatic cancer have an APPR, as shown by elevated levels of CRP, and elevated proinflammatory cytokine levels with higher levels of IL-6 and the TNF receptors compared with controls. Levels of sIL-6R, however, were not significantly elevated. We have previously demonstrated higher levels of CRP and IL-6 in similar groups of patients [5] but had not previously examined TNF or IL-6 receptor levels. sIL-6R has been found to be elevated compared with controls in patients with haematological malignancy [20] and interstitial lung diseases [21].

We also examined the relationship between the APPR and serum levels of IL-6, sIL-6R, sTNF-R55 and sTNF-R75 in patients with advanced pancreatic cancer and found that while IL-6, sTNF-R55 and sTNF-R75 levels were significantly positively associated with the level of the acute-phase response, the sIL-6R level was not. This suggests that, in advanced pancreatic cancer, serum sIL-6R may not be important in conducting the IL-6 signal. The IL-6 bound to sIL-6R may be unavailable for the induction of signalling via gp130 or hepatocytes may be refractory to this mechanism in vivo. In addition, serum levels of sIL-6R may not accurately reflect relevant tissue levels. However, the results of the present study suggest that sIL-6R appears to be a poor indicator of the inflammatory response in pancreatic cancer. Studies of an animal model of autoimmune disease have suggested the presence of elevated sIL-6R levels in association with increasing levels of IL-6 [22], and a weak but significant association was shown between sIL-6R and disease stage in human subjects with haematological malignancy [20]. However, a lack of association between serum sIL-6R and CRP was seen in patients with interstitial lung disease [21] as in the present study.

A close association between levels of sTNF-R55 and sTNF-R75 has previously been shown in sarcoidosis [13] and asthma [14]. An association between TNF receptor levels and disease severity was found in these conditions and also in cancer [23] and severe sepsis [12] but not in a small group of patients with mild psoriasis [24]. Whether the high levels of TNF receptors in this variety of inflammatory conditions represent an attempt by the body to limit cytokine effects by binding TNF or if this binding serves to hold TNF in the circulation resulting in sustained, controlled release remains to be elucidated. It also remains to be seen whether assessment of the levels of TNF receptors may provide a more accurate representation of TNF activity than TNF itself.

Although we have shown significant correlations between CRP concentration and IL-6 and TNF receptors using methods appropriate to the non-parametric distribution of the data there were outliers, particularly among the patients with a relatively low serum CRP. Clearly the APPR is a heterogeneous process involving many proteins in addition to CRP. It may be that the concentration of another protein or an index of values for several proteins will, in the future, provide a more accurate assessment of the acute-phase response and, consequently, a better correlation with other inflammatory mediators.

In the past we were unable to show an association between the acute-phase response and serum levels of IL-6 in patients with pancreatic cancer although a strong association with peripheral blood mononuclear cell IL-6 production was seen [5]. Others have found a relationship between serum levels of IL-6 and survival in lymphoma [25] and weight loss in colon cancer [26]. Previous studies at this centre showing the presence of an APPR to be a strong predictor of poor survival in pancreatic cancer have used a serum CRP level of 10 mg/l or more to define
those patients with an APPR [7]. The use of ELISA techniques in the current study rather than a turbidometric assay has allowed us to examine the relationship of inflammatory mediators to CRP levels substantially below 10 mg/l. These data indicate a clear association between IL-6 and the APPR. It remains a matter of conjecture whether local IL-6 production in the liver is more important in stimulating the APPR than circulating serum levels. It would appear that IL-6, sTNF-R55 and sTNF-R75 are associated with changes in CRP even at relatively low levels and may have a role in determining the level of the APPR in these patients.

In conclusion, this study has shown that the level of the APPR is significantly associated with serum levels of IL-6, sTNF-R55 and sTNF-R75 in patients with pancreatic cancer but there is no such association with sIL-6R. This study suggests that sIL-6R does not have a significant influence on the magnitude of the APPR in pancreatic cancer.

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