Studies on the Contact System of Coagulation during Therapy with High Doses of Recombinant IL-2: Implications for Septic Shock

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Summary

Patients treated with high doses of interleukin-2 (IL-2) because of cancer, develop hemodynamic and vasopermeability changes, that resemble those observed in sepsis. These patients thus provide a unique opportunity to study the early events in the development of septic shock. We analysed the changes that occurred in the contact system of coagulation in plasma from 4 patients, who together received seven 12-day cycles of high doses of IL-2. Levels of factor XII and prekallikrein during the cycles progressively fell to 50 and 30% of their initial levels, respectively, whereas significant increases in plasma factor XIIa-and kallikrein-C1-inhibitor complexes were not observed (in 3 out of 211 samples slightly increased levels of both complexes were found). The reductions in factor XII and prekallikrein were only in part due to protein leakage, since levels were still significantly lower, i.e., 80 and 50%, respectively, when corrected for albumin decreases. Levels of high molecular weight kininogen (HMWK) also decreased during IL-2 therapy, however, this decrease paralleled that of albumin. SDS-PAGE analysis of plasma HMWK did not reveal increased cleavage of this protein. The reduction of factor XII and prekallikrein, corrected for protein leakage, significantly correlated with albumin levels and inversely with daily cumulative weight gain in the patients.

Thus, we demonstrate that factor XII and prekallikrein decrease during IL-2 therapy. As these decreases, already observed after 1 day treatment, were disproportional to that of albumin, a negative acute phase reactant, and correlated with signs of the vascular leak syndrome, we favor the explanation that they reflected activation rather than a decreased synthesis of the contact system proteins. Further studies are needed to substantiate this hypothesis.

Introduction

The contact (or intrinsic) system of coagulation consists of the proteins factor XII (Hageman factor), prekallikrein, high molecular weight kininogen (HMWK), and factor XI (1, 2). Activation of this system is initiated by binding of factor XII to an activating surface (3, 4). Subsequently, factor XII becomes activated (designated as factor XIIa), and cleaves, in the presence of HMWK, prekallikrein and factor XI to kallikrein and factor Xla, respectively (1, 2). Kallikrein in turn cleaves surface-bound factor XII (reciprocal activation) (5) and HMWK with release of bradykinin (1, 2). The major inhibitor of the contact system in plasma is C1-inhibitor (C1-inh), which serine protease inhibitor forms stable complexes with both factor XIIa and kallikrein (6–11).

The precise physiological role of the contact system is unknown. However, it is generally believed that this system is involved in inflammatory reactions (1, 2, 12), since activation products of the contact system increase vasopermeability, induce vasodilatation, and activate granulocytes (12–16). Excessive activation of the system is considered to play a role in the pathophysiology of septic shock (17–21). Plasma levels of prekallikrein and factor XII in sepsis are decreased, most notably in patients with fatal septic shock. These reductions in factor XII and prekallikrein are believed to reflect increased consumption due to activation. Increased plasma levels of activation products of the contact system in septic patients would support this hypothesis. Recently, we measured serial plasma levels of factor XIIa-C1-inh and kallikrein-C1-inh complexes, which reflect activation of the contact system, in a group of 48 patients with sepsis (22). Levels of these complexes appeared to be increased in only 40% of the patients, and in most of these patients only on one occasion, and did not correlate with clinical symptoms including decreased arterial pressure (22). However, mean arterial pressure in these patients with sepsis very significantly correlated with total factor XII as well as with total prekallikrein levels (23), which suggests that contact activation is involved in the development of hypotension in sepsis. Thus, the precise role of the contact system in septic shock remains to be established.

Understanding the role of the contact system in septic shock requires prospective follow-up studies in patients at high risk for sepsis to establish whether plasma levels of activation products of the contact system increase during the development of septic shock, or whether factor XII and prekallikrein decrease disproportionally to albumin. However, these studies are difficult to perform, since they require frequent blood sampling from patients, who in most cases will not develop septic shock. Recently, it has been described that patients who receive high doses of recombinant interleukin-2 (IL-2) as a treatment for malignant diseases, develop hemodynamic alterations and changes in vasopermeability virtually indistinguishable from those present in septic shock (24, 25). Thus, patients who receive high doses of IL-2 provide a unique opportunity to analyse the sequential changes of the contact system that occur during the development of a septic shock-like syndrome. In this investigation we report on a study on the contact system in 4 patients who received 7 cycles of high doses of IL-2.

Patients, Materials and Methods

Patients

Four patients (1 female, 3 male, ages ranging 34–57 years) participated in this study after informed consent. Three of them had advanced

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metastatic melanoma, one suffered from metastatic renal carcinoma. All patients were admitted to the Intensive Care Unit for appropriate management of the anticipated hemodynamic complications. The recombinant IL-2 (Cetus Corporation, Emeryville, CA) therapy consisted of a 12 day period: IL-2 was administered daily on days 1–5 and 8–12, no IL-2 was given on days 6 and 7. IL-2 was administered as a bolus infusion in 30 min with a starting dose of 3 x 10^6 Units per m^2. The dose was gradually increased by 3 x 10^6 U per m^2 every 2–3 days to 12 x 10^6 U per m^2 on days 11 and 12. The dose of IL-2 was not increased and occasionally even lowered when side effects were too severe. Three patients were treated with a second cycle of IL-2 1 month after completion of the first cycle and when all toxicity had fully resolved according to the same regimen except that IL-2 was given as a continuous rather than a bolus infusion. Further details on the patients as well as on studies on their complement system are given elsewhere (26).

Blood Sampling

Blood samples for measurements on the contact system were obtained via arterial lines as described (22). The blood was collected in siliconized tubes that contained Polybrene (0.05%, w/v; final concentration) and EDTA (0.01 M; final concentration) to prevent in vitro activation of the contact (and of the complement) system and processed as described previously (22, 27). Plasma samples were stored (within 60 min after sampling) in aliquots at −70° C until tested.

Analysis of Contact System Proteins

Factor XII and prekallikrein antigen levels in plasma were measured by radioimmunoassays as described (22). In these assays both the zymogenic and the activated species of the proteins are measured. Both assays were specific as was demonstrated by results obtained with plasma samples deficient in either factor XII or prekallikrein (George King Biomedical Inc., Overland Park, KS). Results obtained with samples were compared with levels of factor XII and prekallikrein in a plasma pool prepared by mixing equal volumes of plasma from 31 healthy donors and expressed as percentage of the levels in this plasma pool (22). It is to be noted that in these assays both the zymogens as well as the prekallikrein-C1-inhibitor complexes are detected. HMWK levels were measured with a similar radioimmunoassay procedure, using monospecific polyclonal goat antibodies against the light chain portion of HMWK (22) kindly provided by Dr. Bonno N. Bouma (Department of Haematology, Academic Hospital, Utrecht, The Netherlands). The assay appeared to be specific for HMWK as plasma deficient in HMWK (George King Biomedical Inc.) did not yield a significant response.

Factor XIIa-C1-inhibitor and kallikrein-C1-inhibitor complexes were measured as described (22). With these assays as little as 0.05% activation of either factor XII or prekallikrein in plasma is detected. Intra- and interassay coefficients of variation of the radioimmunoassays described above were less than 11%.

Clotting of HMWK in plasma was assayed by the method of Berrettini et al. (29) with minor modifications. Plasma samples (4 μl) were separated by SDS polyacrylamide (7%; w/v) gel electrophoresis (SDS-PAGE) under non-reducing conditions. Then, proteins in the gels were electrophoretically transferred to nitrocellulose sheets. The sheets were then incubated with 125I-affinity purified goat antibodies against the light chain of human HMWK, and after a washing procedure subjected to autoradiography to visualize the HMWK species. Details of the procedure were as previously described (30). The apparent molecular weight of protein bands was estimated by comparison with the high molecular weight protein markers of Biorad (Richmond, CA). The extent of HMWK cleavage was assessed by comparing the intensities of the bands in patients' plasma with those in pooled normal plasma and plasma maximally activated with dextran sulphate and kaolin (27).

Factor XIIa-C1-inhibitor and kallikrein-C1-inhibitor complexes were measured in plasma samples that were obtained at 6–8 h intervals during the observation period. On the average, 3 samples per day were analyzed from each patient. Levels of factor XII, prekallikrein, HMWK, and of other plasma proteins were assayed in samples that were obtained each day prior to the start of the IL-2 infusion.

Determination of Plasma Proteins

Plasma levels of albumin and immunoglobulin G (IgG) were determined with an automated nephelometer (Behringwerke AG, Marburg, Germany) according to the manufacturer's instructions.

Data Analysis

For statistical analysis of the data the SPSS package was used. Correlation between parameters was assessed by linear regression analysis. A P value lower than 0.05 was considered to represent difference. Differences between parameters at day 1 with those at subsequent days were evaluated by the Wilcoxon-Mann-Whitney (WMW) test.

Results

Clinical and Hemodynamic Parameters

All 4 patients experienced the typical side effects, that are observed after administration of high doses of IL-2 (31). In general, symptoms started about 2–4 h after completion of the IL-2 infusion and were most prominent at the higher doses of IL-2. Only one patient received the highest planned dose (12 x 10^6 U/m^2). All 4 patients developed a septic shock-like syndrome, characterized by an increase in cardiac output, a decrease in vascular resistance, and hypotensive reactions that required therapy with vasoactive drugs (see ref. 26). In addition, the patients exhibited an impressive weight gain due to the formation of peripheral edema. Three patients received a second cycle of high doses of IL-2, 4 weeks after the first cycle. Clinical and hemodynamic symptoms as well as routine laboratory findings during the second cycle were essentially identical to those of the first. Therefore, all data of the 7 cycles of IL-2 therapy in the 4 patients were analysed together.

Contact System Parameters

Levels of prekallikrein, factor XII and HMWK in the patients before the start of the IL-2 cycles are given in Table 1. In particular levels of factor XII varied widely between the patients, and levels during the cycles were therefore related to initial levels, which were arbitrarily set at one. Both factor XII and prekallikrein progressively decreased during IL-2 therapy, to 50 and 30% of their initial levels, respectively (Fig. 1). The differences between levels at day 1 and those at the subsequent days were significant for factor XII as well as for prekallikrein (0.017 >> p < 0.007, WMW-test). Levels of both contact system proteins correlated significantly with each other (r = 0.81, p < 0.0001). In all but three plasma samples neither factor XIIa nor kallikrein-

### Table 1 Contact system proteins in the patients before the IL-2 treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Factor XII</th>
<th>Prekallikrein</th>
<th>HMWK</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st cycle</td>
<td>78</td>
<td>64</td>
<td>71</td>
</tr>
<tr>
<td>2nd cycle</td>
<td>68</td>
<td>61</td>
<td>82</td>
</tr>
<tr>
<td>1st cycle</td>
<td>43</td>
<td>134</td>
<td>81</td>
</tr>
<tr>
<td>2nd cycle</td>
<td>35</td>
<td>101</td>
<td>71</td>
</tr>
<tr>
<td>1st cycle</td>
<td>224</td>
<td>137</td>
<td>96</td>
</tr>
<tr>
<td>2nd cycle</td>
<td>162</td>
<td>131</td>
<td>81</td>
</tr>
<tr>
<td>Median (range)</td>
<td>(71-133)</td>
<td>(74-136)</td>
<td></td>
</tr>
<tr>
<td>Normal values</td>
<td>98</td>
<td>91</td>
<td>102</td>
</tr>
</tbody>
</table>

1Expressed as % of pooled normal plasma.
2Median (range) determined in 31 healthy volunteers.
CI-inhibitor complexes were increased despite the progressive fall observed in factor XII and prekallikrein levels (approximately 3 plasma samples a day were analysed from each patient, the total number of samples analysed being 211). Activation in those three samples was up to 0.85% for factor XII (normal less than 0.05%, ref 22) and up to 1.2% for prekallikrein (normal less than 0.25%).

HMWK levels also decreased during IL-2 therapy, to approximately 75% of the initial level (Fig. 1), though not so markedly as factor XII and prekallikrein. Except for at day 8, HMWK levels at day 2 and subsequent days were significantly lower than those at day 1 (0.048 p>0.0007, WMW-test). Plasma samples obtained from each patient before the start of the treatment and at approximately 4 h after the IL-2 infusion at day 12, were analysed for cleavage of HMWK. In normal plasma (lane 1, Fig. 2) a predominant band of Mr ~130,000 and a minor band with Mr ~110,000 (arrows a and b in Fig. 2, respectively) both representing intact HMWK (32), were observed. In plasma activated with dextran sulphate (lane 2, Fig. 2) or kaolin (lane 3) (27), bands with Mr ~110,000 and ~95,000 (arrow c, Fig. 2) were observed, whereas no bands were observed with HMWK-deficient plasma (lane 4). In none of the samples obtained from the patients was a significant degeneration of HMWK observed (examples are shown in lanes 5–14, Fig. 2).

Levels of Contact System Proteins in Relation to those of Albumin and IgG

The discrepancy between the decrease of factor XII, prekallikrein as well as of HMWK and the absence of significant amounts of activation products could either be due to activation of the contact system and subsequent rapid clearance of activation products from the circulation, or be caused by other processes such as leakage of proteins into the interstitial fluid. To assess the contribution of these processes to the reduction in plasma levels of the contact system proteins, we measured albumin and IgG, 

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**Fig. 1**  Factor XII, prekallikrein and HMWK levels during IL-2 therapy. In each patient, initial levels were arbitrarily set at 1, and levels at subsequent days were related to these. Data shown represent the mean level of the 7 cycles at each day; standard errors for the mean are indicated by bars. Except for HMWK at day 8, levels at day 1 differed significantly from those at subsequent days (WMW-test).

**Fig. 2**  Immunoblot analysis of HMWK during IL-2 therapy. Arrows indicate bands with Mr 130,000 (a), 110,000 (b), and 95,000 (c). Lane 1: normal plasma; lane 2: dextran sulphate-plasma; lane 3: kaolin-plasma; lane 4: kininogen deficient plasma; lanes 5–14: patient's plasma, lanes 6, 9, 11 and 12 before IL-2 therapy, other lanes after. See Materials and Methods for details.
which proteins were assumed to be inert. Both albumin as well as IgG levels progressively declined during IL-2 therapy to 65 and 75% of their initial values, respectively (Fig. 3), the differences between levels at day 1 and those at the subsequent days being significant for either protein (0.03 > p > 0.0007, WMW-test). We then calculated for each sample the ratio between the decrease of the contact system proteins relative to that of albumin and IgG. For this calculation, levels relative to the initial levels, with the latter value being arbitrarily set at one, were used. The ratio between prekallikrein and albumin significantly fell during IL-2 treatment to 0.77 at day 5 and to approximately 0.5 at days 11 and 12 (Fig. 4, 0.017 > p > 0.0007, WMW-test, for the differences between day 1 and subsequent days). Notably, this ratio significantly further decreased during the second week (0.017 > p > 0.0007, WMW-test, for the differences between the ratio at day 8 and those at subsequent days). Thus, prekallikrein fell to approximately 50% of its initial level even when corrected for the decrease in albumin. Also the ratio between factor XII and albumin fell (Fig. 4), and except for the ratio at day 8, also this decrease was significant (0.0028 > p > 0.0007, WMW-test). Similar results were obtained with ratios of factor XII and prekallikrein to IgG (data not shown). Thus, the decrease of factor XII and prekallikrein during IL-2 therapy was disproportional to that of albumin and IgG. In contrast, the ratio between HMWK and albumin did not decrease during the IL-2 therapy, but rather increased (Fig. 4), which increase was significant during the second week (0.017 > p > 0.0008, WMW-test).

**Relations of Levels of Contact Proteins to Signs of the Vascular Leak Syndrome**

The decrease in prekallikrein levels (corrected for the decrease in IgG) inversely correlated with the increase in weight of the patients (Fig. 5). A similar correlation was found when levels were corrected for the decrease in albumin (r = –0.42, p = 0.0004). In addition, prekallikrein levels (corrected for IgG) significantly correlated with albumin levels (Table 2). Similar correlations were found for factor XII but not for HMWK (Table 2). No significant correlations were found between levels of the contact system proteins and hemodynamic parameters including systemic vascular resistance and arterial blood pressure.

**Discussion**

In this study we analysed the changes in the contact system that occurred in patients who developed a septic shock-like syndrome

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**Fig. 3** Albumin and IgG levels during IL-2 therapy. Results are given in a similar way as is done in Fig. 1. Levels at day 1 differed significantly from those at subsequent days (WMW-test)

**Fig. 4** Factor XII, prekallikrein and HMWK levels corrected for protein leakage during IL-2 therapy. In each patient, levels of factor XII, prekallikrein and HMWK at the indicated days were divided by albumin levels at the same day, with initial levels of each protein being set at 1, and levels at subsequent days being related to initial levels. Results are given as the mean ratio for the 7 cycles at each day. Bars indicate standard errors of the mean. Except for factor XII at day 8, levels of factor XII and prekallikrein at day 1 differed significantly from those at subsequent days (WMW-test). The increases in HMWK at days 8–12 also were significant.
during therapy with high doses of IL-2. Our results indicated that factor XII and prekallikrein progressively decreased during this therapy, and that the decline of these contact proteins was disproportionate to that of IgG and albumin. Thus, this decrease was not simply due to protein leakage from the circulation, since it would then be expected to be proportional to that of IgG and albumin.

We made several efforts to demonstrate increased plasma levels of activation products of the contact system in the patients to support the view that activation had occurred. Despite the use of assays, that are capable of detecting as little as 0.05% activation of factor XII and prekallikrein in plasma (22), we only found evidence for activation in 3 out of 211 plasma samples tested. Determination of ratios between functional and antigenic levels has been used to assess activation of contact system proteins (17). We did not measure functional levels of factor XII and prekallikrein in our patients as Colman and coworkers elegantly demonstrated that the discrepancy between functional and antigenic levels in patients with typhoid fever was due to complexation of kallikrein to C1-inhibitor (17), and we specifically measured kallikrein- (and factor XIa-) C1-inhibitor complexes in addition to measuring antigenic levels. In additional experiments not shown here we also assessed cleavage of factor XII and prekallikrein by an immunoblotting technique which resembles that used to assess cleavage of HMWK. With these methods, which in our hands allow the detection of approximately 5–10% activation of factor XII and prekallikrein in plasma, we found no evidence for activation of either contact protein in the IL-2 treated patients indicating that both proteins for the major part circulated aszymogens. Serial measurements in patients with sepsis revealed a maximal half-life time of clearance of 50 min for factor XIa- as well as for kallikrein-C1-inhibitor complexes (22). Turnover studies with thrombin-antithrombin III and with elastase-α1-antitrypsin (33, 34) suggest that the actual clearance in vivo might be even faster. Therefore, we presume that the lack of detection of activation of factor XII and of prekallikrein in the patients may have been due to the rapid clearance of factor XIa- and kallikrein-C1-inhibitor complexes in vivo.

Griffin and coworkers have proposed another method for detecting contact activation in plasma, i.e., by assessing cleavage of HMWK (29). With this method activation of the contact system in patients with hereditary angioedema was demonstrated (29, 35), and this is also our experience (M. Cugno et al., manuscript in preparation). In the IL-2 treated patients, who all developed a septic shock-like syndrome, we did not observe significant cleavage of HMWK (see Fig. 2) which is in agreement with observations by Schmaier et al. (36), who found no evidence for HMWK cleavage in patients with septic shock. In addition, the decrease in plasma levels of HMWK during the IL-2 treatment paralleled that of albumin the first week of the cycle, and even significantly became elevated during the second week (Fig. 4). These findings may suggest that HMWK was not activated during the IL-2 treatment. However, at this moment we cannot exclude the possibility that some cleavage of HMWK had occurred in the patients, and that the cleaved HMWK formed had escaped detection either because of a rapid clearance and or binding to activators, or because of a too low sensitivity of the assay (36). The significant rise in levels of HMWK, when corrected for albumin levels, during the second week of IL-2 treatment might be explained by the differences in molecular weight between albumin (MW 67,000) and HMWK (MW 120,000) and the subsequent differences in leakage. However, this seems unlikely as HMWK also was significantly increased when compared with IgG (MW 160,000). The increase in HMWK presumably points to acute phase behaviour of this protein. Therapy with high doses of IL-2 induces an acute phase response in patients. In rats, a protein homologous to HMWK is synthesized as a major acute phase reactant (37). A thorough analysis of HMWK synthesis during acute phase reactions in humans has to our knowledge not been performed. However, Colman and coworkers have suggested that the striking rise of HMWK they observed in typhoid fever, reflects its acute phase behaviour (17). In agreement herewith, in preliminary experiments with the liver cell-line Hep G2, which is frequently used to study the synthesis of acute phase

![Weight gain (% of initial)](attachment)

Fig. 5 Relationship of prekallikrein, corrected for IgG, to daily cumulative weight gain. Levels of prekallikrein were divided by IgG levels in a similar way as is done in Fig. 4, to correct for protein leakage. Daily cumulative weight gain is expressed as percentage of the initial weight of each patient.

| Table 2 Relation of contact system proteins to signs of the vascular leakage syndrome |
|---------------------------------|-----------------|-----------------|
|                                | Weight increase | Albumin         |
| Prekallikrein                  | -0.47 (p < 0.0001) | 0.58 (p < 0.0001) |
| Factor XII                    | -0.38 (p = 0.0024) | 0.34 (p = 0.005) |
| HMWK                           | 0.03 (not sign.) | 0.03 (not sign.) |

1 Daily cumulative increase (% of initial weight).
2 Decrease relative to the decrease of IgG, initial ratio arbitrarily set at 1.
3 Coefficient of correlation.
proteins (38, 39), we observed that HMWK was produced as an acute phase protein. Thus, an increased catabolism of HMWK due to activation might have been masked in our patients by an increased synthesis.

Despite several efforts we did not detect increased levels of activation products of the contact system in our patients. Therefore, a reduced synthesis of factor XII and prekallikrein cannot be excluded definitely as the cause for the decreases of both proteins. However, for several reasons we think that reduced synthesis does not explain our findings. Firstly, levels of factor XII and prekallikrein already started to decrease at day 1 (Figs. 1 and 4), which seems rather rapid in case of reduced synthesis. Secondly, reduced levels of factor XII and prekallikrein have been shown to occur in liver cirrhosis pointing to the liver as the major site of synthesis for both proteins. Protein synthesis of the liver in general was not effected in our patients as for example levels of α1-antitrypsin were even increased (data not shown). In addition, the decreases of factor XII and prekallikrein exceeded that of albumin which synthesis in our patients presumably was reduced as it is a negative acute phase protein. Thirdly, the decreases of factor XII and prekallikrein correlated with signs of the vascular leak syndrome (Table 2, Fig. 5). Considering the effects of bradykinin on vascular permeability (13, 14) these observations are more consistent with activation of the contact system than with reduced synthesis.

An increased vasopermeability clinically manifests itself as oedema formation and weight gain (31). It causes leakage of plasma proteins into the interstitial fluid, which amongst others results in low plasma levels (40). We observed significant correlations between the decreases in factor XII and prekallikrein (corrected for IgG decrease) and the increase in weight and the decrease in albumin in our patients (Table 2, Fig. 5). Notably, the vascular leakage syndrome got worse during the second week of therapy, as judged by weight gain. During this period both factor XII and prekallikrein still further decreased, being significantly lower at day 9 and subsequent days compared with levels at day 8 (Fig. 4). These observations suggest that contact activation plays a role in the development of the vasopermeability changes.

Several mechanisms have been proposed to explain the increased vasopermeability that is observed during therapy with high doses of IL-2. Adherence of activated killer cells to endothelial cells under influence of cytokines that are induced by IL-2, may result in damage of the latter cells (41–43). In addition, activation of the complement system presumably is involved as plasma levels of the anaphylatoxins, which can increase vasopermeability and activate neutrophils (44, 45), are elevated in patients who developed the vascular leakage syndrome during IL-2 therapy (26, 46). Activated neutrophils that adhere to endothelial cells, do damage to these latter cells by the release of oxygen radicals and elastase (47, 48). We have observed that plasma levels of elastase and lactoferrin, which both are released by activated neutrophils, increased during IL-2 therapy (J. W. Baars et al., manuscript in preparation). Thus, also activated neutrophils may contribute to the changes in vascular permeability.

The results of this study suggest that the contact system also is involved in this process, either directly because of effects of its activation products on vasopermeability (13, 14), or indirectly via their effects on neutrophils (15, 16). Thus, the pathogenesis of the vascular leakage syndrome induced by IL-2 therapy appears to be complex and involves activation of multiple effector mechanisms.

IL-2 therapy not only may induce an increase in vasopermeability, but also may be accompanied by severe hypotensive reactions (24, 25). Bradykinin, which is generated during contact activation, is a very potent inducer of vasodilatation and hypotension (49). We found no correlation between mean arterial pressure and factor XII and prekallikrein levels in the IL-2 treated patients. However, we do not think that this excludes a role for contact activation in the development of hypotensive reactions during IL-2 therapy, since the patients that were studied, were treated with vasopressor drugs and this undoubtedly influenced the correlation with mean arterial pressure (26).

A detailed analysis of the changes of the contact system in relation to the alterations in hemodynamic parameters in patients who receive IL-2, is needed to appreciate the exact role of contact activation, if any, in the IL-2 induced hypotension. Alternatively, the effect of potent inhibitors of activated contact proteins such as the Pittsburgh mutant of α1-antitrypsin (50) could be studied in animals who receive high doses of IL-2.

The clinical symptoms, biochemical, hematological and hemodynamic changes that are induced by high doses of IL-2 remarkably resemble the events that occur in sepsis (24, 25, 31). Also with respect to involvement of inflammatory mediators such as cytokines (51–53), neutrophils (54, J. W. Baars et al., manuscript in preparation), and complement (26, 46, 55), both syndromes appear to be similar. In septic shock, levels of factor XII and of prekallikrein are very low, although definite proof that this is due to activation rather than to protein leakage from the circulation is still lacking. Here we demonstrated that both factor XII and prekallikrein decrease disproportionally to albumin and IgG in patients who received high doses of IL-2, which suggests that activation of the contact system has occurred. Therefore, because of the remarkable resemblance between sepsis and the syndrome that is induced by therapy with high doses of IL-2, we propose that the low levels of factor XII and of prekallikrein in sepsis at least in part reflect activation of the contact system.

REFERENCES


