Complement Activation in Cancer Patients Undergoing Immunotherapy With Interleukin-2 (IL-2): Binding of Complement and C-Reactive Protein by IL-2–Activated Lymphocytes

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Plasma samples from cancer patients undergoing immunotherapy with high-dose recombinant interleukin-2 (IL-2) were obtained over a 5-day course of treatment and assayed by radioimmunoassay or enzyme-linked immunosorbent assay for the complement degradation products, C3a, iC3b, Ba, Bb, C4d, and SC5b-9. In the majority of patients, pretreatment C3a, Ba, Bb, and SC5b-9 plasma levels were comparable with those measured in normal donor plasma. However, by the end of the 5-day treatment course, C3a levels had increased 15.6-fold. In several patients, peak concentrations of C3a were as high as those reported in patients with sepsis or burn injury. Plasma levels of alternative pathway components Ba and Bb also increased, 8.0- and 5.0-fold, respectively, during IL-2 treatment. Likewise, levels of one of the terminal complexes, SC5b-9, increased 5.0-fold and the plasma C4d and iC3b concentrations increased 4.8- and 2.9-fold, respectively, by the fifth day of treatment. To determine whether activated lymphocytes participate in IL-2–induced complement activation, peripheral blood mononuclear cells (PBMC) obtained from IL-2 recipients before and 5 days after beginning therapy were reacted with monoclonal antibodies (MoAbs) against C3c and the terminal complement complex SC5b-9. Dual-color cytofluorographic analysis showed that within the CD3(+) population, the percentage of cells binding the anti-C3c and anti-SC5b-9 MoAbs increased 6.2-fold and 5.1-fold, respectively, by day 5. The anti-C3c MoAb also bound to CD3(+) cells stimulated in vitro with IL-2 and then exposed to serum. Moreover, fluid-phase iC3b was generated from purified C3 by PBMC activated in vitro with IL-2, but not by unstimulated cells. Serum levels of C-reactive protein (CRP) are markedly elevated in patients undergoing IL-2 immunotherapy. This hepatic acute phase reactant has been shown to activate the classical pathway when bound to cell surfaces. Because levels of the classical component C4d increase markedly during IL-2 treatment, we sought to determine if CRP became bound to PBMC during IL-2 treatment and found that during therapy, the percentage of CD3(+) cells reactive with an anti-CRP MoAb increased from less than 2% to greater than 18%. When PBMC were activated with IL-2 in vitro and then exposed to exogenous CRP, greater than 20% of the CD3(+) cells reacted with the anti-CRP MoAb. Moreover, when autologous serum and purified CRP were added to cultured IL-2–primed PBMC, the percent of CD3(+) cells reactive with the anti-C3c antibody increased as a function of the CRP concentration. These results suggest that T lymphocytes activated in vitro with IL-2 or in vivo during a course of IL-2 immunotherapy bind complement and that this binding is amplified by the high plasma CRP concentrations induced by IL-2 administration. The significance of these findings and their relationship to the IL-2–induced vascular leak syndrome are discussed.

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HIGH-DOSE RECOMBINANT interleukin-2 (IL-2) is currently being evaluated in clinical trials as an immunotherapeutic agent against a variety of malignancies.1,8 Although tumor regression occurs in 20% to 30% of patients with malignant melanoma or renal cell carcinoma, the antineoplastic potential of IL-2 is limited by its severe toxicity. IL-2 recipients frequently develop chills, fever, nausea, and hypotension as well as hepatic and renal dysfunction. In addition, most patients develop a generalized capillary leak syndrome manifested by serosal effusions and peripheral and pulmonary edema. Progressive abnormalities in pulmonary function are a universal accompaniment of IL-2 therapy and may result in hypoxia and the appearance of pulmonary infiltrates on chest radiographs. These potentially life-threatening complications appear in the setting of normal or low left atrial pressures and are therefore more likely to be due to increased vascular permeability rather than fluid overload.2,5

The severity and diversity of symptoms and the multiple organ dysfunction associated with IL-2 toxicity are similar to those that accompany severe burn injury and sepsis, both of which are associated with complement activation and anaphylatoxin formation. In patients with sepsis or severe burns, the increase in C3a levels is temporally linked to the development of an acute respiratory distress syndrome (ARDS).1,10-13 Thijs et al14 recently reported an elevation in plasma C3a levels during IL-2 therapy that correlated with the severity of the vascular leak syndrome. In the present study, we confirmed these findings and, in addition, showed high plasma concentrations of other complement degrada-

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activate complement. Using antibodies directed against C3 fragments and the terminal complement complex, we found that, during IL-2 therapy, these complement fragments were bound to the surface of circulating CD3(+) lymphocytes. Moreover, CD3(+) cells from healthy donors stimulated in vitro with IL-2 and then incubated with autologous serum also reacted with the anti-C3e antibody.

We also postulated that the acute-phase C-reactive protein (CRP) may be involved in IL-2-induced complement activation. This conjecture was based on the high plasma levels of CRP in IL-2-treated patients and the results of previous studies showing that CRP immobilized on a cell surface can bind C1 and C4, thereby initiating the activation of the classical pathway of complement. When PBMC obtained on day 5 of IL-2 therapy were reacted with an anti-CRP antibody, over 18% of the CD3(+) cells bound this antibody. Moreover, over 20% of the CD3(+) cells stimulated in vitro with IL-2 and then exposed to purified CRP reacted with this antibody. In addition, binding of C3 fragments by T cells stimulated with IL-2 was increased in the presence of exogenous CRP. Our data thus suggest that IL-2–stimulated CD3(+) cells can activate complement and that CRP augments this activation. This process may contribute to the capillary leak syndrome and other inflammatory events associated with IL-2 immunotherapy.

MATERIALS AND METHODS

Clinical specimens. Blood samples for assays of complement degradation products were collected in EDTA-containing venoject tubes 2 to 3 hours before and at various time points after initiating IL-2 treatment. Day 5 samples were obtained 5 to 6 hours after the morning IL-2 dose. The plasma was separated by centrifugation within 15 minutes of venipuncture and frozen at −70°C until assayed. The IL-2 used in these studies was provided by Cetus (Emeryville, CA) and Hoffman-La Roche (Nutley, NJ). The patients providing the serial blood samples for this investigation received intensive IL-2 therapy at 6.0 × 10⁶ IU/kg (1.0 mg = 1.8 × 10⁶ IU) every 8 hours by intravenous bolus injection to a maximum of 14 doses. The treatment was repeated after a 9-day respite; however, the studies on complement activation were performed only during the first week of treatment. Although the number of patients providing material for the individual complement assays varied, all assay results are displayed in the appropriate figures. All patients participating in these studies had a disseminated malignancy (melanoma or renal cell carcinoma) for which no alternative therapy was available. The clinical trials pertinent to this investigation were approved by the sponsoring agency and the human investigation review committee at the New England Medical Center and all study participants gave informed consent.

Measurement of circulating complement degradation products. Concentrations of iC3b, C4d, Ba, Bb, and SC5b-9 in plasma and culture supernatants were measured by enzyme-linked immunosorbent assay (ELISA; Quidel, San Diego, CA). All components were measured by the double-sandwich antibody method using monoclonal and polyclonal antibodies. Plasma C3a des-arg concentrations were measured by radioimmunossay (RIA; Amersham, Chicago, IL). Statistical values and fold increases were expressed as the mean ± the standard deviation. Data were analyzed by paired t-tests using log-transformed data.

Cytological analysis of PBMC obtained during IL-2 therapy. PBMC were isolated from the heparinized blood of patients receiving IL-2 by centrifugation over a Ficoll-Hypaque density gradient. The mononuclear cell layer was removed, washed twice in phosphate-buffered saline (PBS) and then suspended in PBS containing 0.1% bovine serum albumin (BSA) and 0.1% azide. The cells were then transferred to polystyrene tubes, adjusted to a density of 5 × 10⁷ cells/100 μL and then incubated for 30 minutes on ice with 8 μL of a monoclonal antibody (MoAb) to either C3e or SC5b-9 (Quidel). The anti-C3e MoAb detects C3e determinants present in the native C3 molecule as well as in its cleavage products iC3b and C3b, while the anti-SC5b-9 MoAb binds to various forms of the terminal complement complex. These forms include the SC5b-9 complex, which is nonlytic due to the presence of the S protein, the sublytic unit SC5b-9, which can initiate the formation of nonlytic channels in the cell membrane, and the membrane attack complex, which forms before cell lysis. Some samples were reacted with 15 μL of an MoAb against native CRP (a gift from Dr. Heary Gewurz, Rush Medical College, Chicago, IL) or with 15 μL of a 1:1,000 dilution of an MoAb against decay-accelerating factor (DAF) (a gift from Dr Victor Nussenzweig, New York University). All samples were then washed twice in PBS-BSA-azide and reacted with a fluorescein isothiocyanate (FITC)-conjugated goat F(ab′), antimurine IgG antibody (Tago, Burlingame, CA) for an additional 30 minutes on ice. The cells were subsequently reacted with a phycoerythrin-conjugated anti-Leu 4 (CD3) antibody (Becton Dickinson, Sunnyvale, CA) for 30 minutes on ice and then washed. Murine Ig (Sigma, St Louis, MO) followed by an FITC-conjugated goat F(ab′), antimurine IgG antibody, the second-step antibody by itself, and FITC-conjugated IgG1 and IgG2 were used as controls. All samples were stored in PBS-BSA-azide buffer containing 0.1% formalin at 4°C until analysis with an Epics 541 FAC (Coulter Electronics, Hialeah, FL). Gates were set to exclude nonviable cells and debris.

C3 fixation by CD3(+) cells stimulated with IL-2 in vitro. PBMC were isolated from the heparinized blood of healthy donors as described above, washed in PBS, and then resuspended in X-Vivo 10 serum-free media (Whittaker Bioproducts, Walkersville, MD) at a concentration of 2 × 10⁶ cells/mL in the presence or absence of 1,000 U/mL IL-2. The cells were cultured in polystyrene flasks for 48 hours at 37°C in 5% CO₂ after which the nonadherent cells were removed, washed twice with Hanks’ Balanced Salt Solution (HBSS) and then resuspended at a density of 2 × 10⁶ cells/300 μL in GVBS (0.1% gelatin in veronal buffered saline, pH 7.4, with 1 mmol/L MgCl₂ and 0.15 mmol/L CaCl₂) containing 30% autologous serum. These samples were then incubated at 37°C for 50 minutes. In some experiments, purified CRP was added to the serum and samples incubated on ice for 50 minutes. The cells were then washed repeatedly, resuspended at a density of 5 × 10⁶ cells/mL in HBSS buffer containing 0.1% BSA and 0.1% azide, and then reacted on ice with antibodies to C3e, SC5b-9, or CRP. The samples were then exposed to the FITC-conjugated goat F(ab′)₃, antimurine antibody and phycoerythrin-conjugated anti-Leu 4 MoAb as described above.

To determine whether C3 is fragmented by IL-2–activated cells, resting and IL-2–activated nonadherent PBMC were adjusted to a density of 2 × 10⁶ cells/300 μL and incubated for 50 minutes at 37°C with purified C3 (14 μg) (Diamedix, Miami, FL) in GVBS. The supernatants were then assayed for iC3b by ELISA.

RESULTS

Measurement of circulating complement degradation products. Complement activation during immunotherapy with IL-2 was assessed by measuring the levels of various classical and alternative pathway breakdown products in
plasma samples obtained sequentially during a 5-day course of treatment. Before treatment, plasma concentrations of C3a, Ba, Bb, and SC5b-9 were comparable with those measured in normal donors (Fig 1). Conversely, C4d and iC3b levels were frequently higher in patients than in healthy controls. Although the levels of these breakdown products remained unchanged during the first few hours of IL-2 treatment, they were consistently elevated at 24 hours in the patients in whom levels were determined (data not shown). However, maximal levels were detected on day 5 in these patients and in all other patients subsequently studied. As shown in Fig 1, C3a levels increased 15.6-fold ± 15.2-fold from 0.3 ± 0.1 to 4.7 ± 6.4 μg/mL by day 5 (P < .03). The C3a level remained unchanged in only one patient. Two patients had day-5 values of 6 and 17 μg/mL, respectively, which are comparable with those reported in the plasma of severe burn victims.8 Plasma levels of iC3b were 10.7 ± 7.3 μg/mL before treatment and increased 2.9-fold ± 1.4-fold to 27.8 ± 14.4 μg/mL by day 5 (P < .001).

Plasma concentrations of alternative pathway degradation products also increased during IL-2 treatment. Ba values increased 8.0-fold ± 2.7-fold from 0.48 ± 0.19 to 3.64 ± 1.4 μg/mL by day 5 (P < .001). Likewise, Bb levels increased 5.0-fold ± 3.3-fold from 0.1 ± 0 to 0.5 ± 0.38 μg/mL (P < .04) (Fig 1). Pretreatment plasma levels of the classical pathway component, C4d, were often higher in cancer patients than in normal individuals; however, concentrations of this component are known to vary widely even among healthy individuals (unpublished data, J.D.T.). Despite the fact that both the pretreatment and day-5 plasma levels of C4d consistently fell within the normal range reported for the ELISA (<8.5 μg/mL), the plasma levels increased 4.8-fold ± 3.5-fold from a mean of 1.3 ± 1.5 μg/mL before treatment to 7.0 ± 3.2 μg/mL by day 5 (P < .001).

C5a is rapidly cleared from the circulation and is not readily detected in the plasma.21 However, plasma levels of the terminal complex, SC5b-9, are a reliable index of C5a production in vivo because both components are generated by the cleavage of C5. The plasma concentrations of SC5b-9 were within the normal range before treatment and increased 5.0-fold ± 2.6-fold from 0.38 ± 0.09 μg/mL before therapy to 1.8 ± 0.75 μg/mL on day 5 (P < .001) (Fig 1). These levels are within the range routinely measured in the plasma of patients with ARDS from sepsis or trauma.22

C3 fragmentation in vitro by IL-2-activated PBMC. Ramos et al previously showed that alloantigen-activated T cells are capable of fragmenting C3 in vitro.13 Klein et al reported essentially identical data with lectin-stimulated
lymphocytes. To determine if IL-2 would provide an activating signal similar to that induced by exposure to concanavalin A (Con A) or an alloantigen, PBMC were cultured for 48 hours in medium alone or medium containing 1,000 U/mL IL-2, the nonadherent cells removed, washed, and then incubated at a density of $2 \times 10^6$ cells/300 μL in GVBS containing purified C3. The culture supernatants were then assayed for iC3b by ELISA and the results expressed as the percentage of C3 fragmented. As shown in Fig 2, the generation of fluid-phase iC3b was critically dependent on the state of activation of the cells in that up to 50% of the exogenous C3 was cleaved by the IL-2-primed nonadherent PBMC, whereas no C3 fragmentation was detectable in unstimulated cultures.

**Binding of complement by PBMC during IL-2 therapy.** The C3 fragmentation by alloantigen-activated T cells reported by Ramos et al and Klein et al was not only manifested by the conversion of C3 to C3b/iC3b but also by the fixation of C3 fragments to the cell surface, which was shown using a fluoresceinated anti-C3c antibody. Because IL-2-primed PBMC generate iC3b in vitro, we sought to determine the extent of complement fixation to the surface of PBMC obtained during the course of IL-2 treatment. To this end, PBMC were obtained before and on day 5 of therapy and reacted with MoAbs against C3c and SC5b-9. Dual-color analyses were performed in an effort to identify the PBMC subsets reactive with the MoAbs and specifically to determine whether T lymphocytes activated in vivo by IL-2 administration could fix complement. Such analyses also served to eliminate the contribution of monocytes, which synthesize complement, to the pool of PBMC reactive with the anti-C3c and anti-SC5b-9 antibodies.

In 8 of the 10 patients studied, the percentage of CD3(+) cells reactive with the anti-C3c antibody increased during the IL-2 treatment. Among these individuals, the percentage increased 6.2-fold ± 3.9-fold from a mean of 5.2% ± 6.6% before treatment to 20.5% ± 13.7% by day 5 ($P < .001$) (Fig 3A). Most of the patients had only a few C3c(+) T cells before treatment. However, the T cells from two patients were greater than 10% positive for C3c. Consistent with these data suggesting ongoing complement activation in these patients, both had high pretreatment plasma levels of iC3b (Fig 1). The patient with the largest percentage (33%) of circulating C3c(+) T cells before treatment had a pretreatment iC3b level of 16 μg/mL, the second highest level recorded in our survey of pretreatment plasma. Although the basis for the complement activation in this patient is unclear, he was persistently febrile ($>38.5^\circ C$) without an obvious infection, suggesting that he may have had an ongoing immune response to his tumor before receiving exogenous IL-2.

While most patients had fewer than 7% SC5b-9(+) T cells before beginning therapy, the two patients with high pretreatment percentages of C3c(+) T cells each had greater than 15% T cells positive for SC5b-9. In six of nine patients examined, the percentage of CD3(+) cells that bound the anti-SC5b-9 antibody increased 5.1-fold ± 3.8-fold from a mean of 11.5% ± 12.4% before treatment to 30.5% ± 13.7% by day 5 (Fig 3B).

When the PBMC obtained on day 5 were sorted by size and complexity, only the larger, presumably activated, CD3(+) cells were found to react with the anti-C3c and anti-SC5b-9 MoAbs. This observation is consistent with the aforementioned results of Ramos et al in which the majority of alloantigen-primed T cells reactive with an anti-C3c antibody were found to be CD25(+). The results of our cytofluorographic analyses are shown in Fig 4. In the population corresponding to small cells (area 1), 38% of the PBMC were CD3(+) T cells. Of these T cells, none were positive for C3c or SC5b-9. In area 2, which contains complex large cells (including monocytes), 8% were CD3 positive and of these, 62% were C3c(+) and 53% SC5b-9(+) T cells. In area 3, which contains the less complex large cells, 18% were CD3(+) and 66% of these T cells were C3c(+) and 42% SC5b-9(+) T cells. Related experiments designed to determine the extent of complement fixation to CD56(+) natural killer (NK) cells were not successful because of the low number of NK cells present in the circulation on day 5 of treatment.

**Role of endogenous CRP in IL-2–induced complement activation.** During IL-2 therapy, serum concentrations of the hepatic acute-phase protein CRP increase from undetectable levels to as high as 750 μg/mL. Although the precise physiologic functions of CRP have not yet been identified, it has been shown to activate the classical pathway of complement when bound to cell membranes. Because plasma levels of the classical component C4d increase markedly after IL-2 administration (Fig 1), we sought to determine if CRP is bound by CD3(+) lympho-
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Fig 3. Fixation of C3c and SC5b-9 by CD3(+) cells during IL-2 therapy. PBMC were obtained from patients treated with IL-2 before beginning treatment and after 5 days of therapy. The PBMC were reacted with an anti-C3c MoAb (A) or anti-SC5b-9 MoAb (B), followed by a fluorescein-conjugated goat antimurine IgG and a phycoerythrin-conjugated anti-Leu 4 MoAb. The percentage of CD3(+) cells reacting with the anti-C3c and anti-SC5b-9 MoAbs was determined by dual-color cytofluorography. The two patients with greater than 10% C3c(+) T cells before initiating treatment (designated with an asterisk) were the ones with the high percentage of pretreatment anti-SC5b-9 MoAb(+) CD3(+) cells and were among the three identified in Fig 1 (*) as having elevated pretreatment plasma iC3b levels.

cytocytes activated during the course of IL-2 treatment. To this end, serial PBMC preparations were obtained and analyzed cytofluorographically using an anti-CRP MoAb. The percent of CD3(+) cells reactive with the anti-CRP MoAb increased 10.2-fold ± 6.7-fold during treatment with IL-2 from a mean of 1.2% ± 1.4% before treatment to 18.3% ± 11.1% by day 5 (P < .01) (Fig 5).

In a parallel set of experiments, PBMC were activated with IL-2 in vitro and then tested for their ability to bind exogenous CRP. The addition of CRP to the cultured cells gave rise to a concentration-dependent increase in the percentage of T cells reactive with the MoAb. At the highest concentration of CRP tested (340 µg/mL), 21.2% ± 10.3% of the CD3(+) cells bound the anti-CRP MoAb (Fig 6). CD3(+) cells not cultured with IL-2 did not bind exogenous CRP (data not shown).

To determine if this association with CRP affected the ability of IL-2-primed PBMC to fix complement in vitro, serum was added to the cultured cells in the presence of increasing concentrations of CRP and the percentage of CD3(+) cells reactive with anti-C3c and anti-SC5b-9 antibodies subsequently determined. In the absence of exogenous CRP, 22.4% ± 12% of the CD3(+) cells stimulated in vitro with IL-2 for 48 hours were reactive with the anti-C3c antibody, whereas at 340 µg/mL CRP (still within the range detected in the serum of IL-2 recipients), 42% ± 16% were positive (Fig 7). The amount of anti-C3c MoAb bound per cell, as determined by the intensity of fluorescence staining, also increased in proportion to the CRP concentration (data not shown). The addition of CRP to unstimulated cells did not affect their reactivity with the anti-C3c MoAb.

Corroborative experiments measuring fluid-phase iC3b generation by PBMC stimulated in vitro with IL-2 and then exposed to CRP were not feasible because the serum used as a source of classical pathway components contains high background levels of iC3b.

While the addition of CRP to IL-2 stimulated PBMC increased the binding of C3 fragments to CD3(+) cells, the binding of the terminal complement complex was not
Fig 5. Binding of CRP by CD3(+) cells during IL-2 therapy. PBMC were obtained from cancer patients before treatment and on day 5 and reacted with a monoclonal anti-CRP antibody, followed by an FITC-conjugated antimouse IgG antibody, and finally with a phycoerythrin-conjugated anti-Leu 4 MoAb.

affected. These results support other studies that have shown that CRP augments the initial steps of classical pathway but has no effect on terminal pathway activation.24,25

Expression of DAF on PBMC. To determine if the fixation of complement to the PBMC of IL-2–treated patients was due to an IL-2–induced reduction in the cell surface expression of DAF, an inhibitor of C3 and C5 convertases,26 PBMC were isolated at various time points during the treatment course and reacted with an anti-DAF MoAb. Between 85% and 95% of unfractionated PBMC and 65% and 75% of CD3(+) cells were found to express DAF both before and on day 5 of IL-2 therapy (data not shown). The intensity of staining with the anti-DAF antibody also remained unchanged, suggesting that the distribution and quantity of DAF on PBMC was not affected by the intravenous administration of IL-2.

DISCUSSION

This investigation has shown that the administration of high-dose recombinant IL-2 to cancer patients induces systemic complement activation resulting in high plasma levels of classical and alternative pathway cleavage products and the fixation of C3b-derived fragments to the surface of PBMC. The physiologic consequences of these findings are unclear at present. However, several of the complement cleavage products generated during IL-2 treatment are known to affect vascular permeability28 and reticuloendothelial function27 and therefore may be responsible for some of the side effects of IL-2 therapy. Of the complement byproducts generated during IL-2 treatment, C3a and C5a (the latter reflected by SC5b-9 production) are of the most potential significance to IL-2–treated patients because these factors are believed to be involved in the pathogenesis of ARDS, a frequent complication of IL-2 therapy.7,9,11 In fact, C3a levels in IL-2–treated patients are often in the range measured in the plasma of patients with severe burns or sepsis, who are prone to develop ARDS.7,12–14 Patients undergoing IL-2 treatment develop a profound defect in neutrophil chemotaxis that may predispose to bacterial infection.20 The C3 cleavage product iC3b has been shown to inhibit neutrophil activity in vitro.20 Likewise, C5a has been implicated in the neutrophil chemotactic defect observed in burn patients.20 Therefore, it is possible that iC3b and C5a, both of which are present in high concentrations in the plasma of IL-2 recipients, may play a role in the impairment of neutrophil function associated with IL-2 therapy.

Up to 20% of the CD3(+) PBMC obtained during a
course of IL-2 treatment are reactive with an anti-C3c antibody, suggesting the fixation of C3b and/or iC3b to the T-cell surface. This reactivity was restricted to CD3(+) cells with a light scattering profile characteristic of a large and complex cell population, indicating an association between activation and the ability to fix complement. In fact, the small uniform cells corresponding to area 1 in Fig 4 were completely unreactive with the anti-C3c MoAb. These results are similar to those of Ramos et al, who noted a correlation between the C3 fixation by alloantigen-primed T cells and their expression of the IL-2Ra chain CD25.13 Although the C3-specific receptors CR1, CR2, and CR3 are widely distributed on PB cell membranes, these receptors primarily bind soluble dimerized C3 fragments or fragments immobilized on a cell surface and their affinity for monomeric fluid-phase cleavage products is very low.14 It therefore appears unlikely that the binding of C3 fragments to CD3(+) cells could be attributed to passive adsorption of fragments onto complement receptors. However, because a subpopulation of T cells has been shown to express CR1,15,16 we investigated the prospect that such CR1(+) cells could bind C3 fragments by incubating unstimulated normal donor PBMC with an acute-phase serum containing high levels of iC3b. The cells were then washed and analyzed cytofluorographically. These cells failed to bind the anti-C3c antibody, indicating that receptor-mediated adsorption of C3 fragments by resting CD3(+) cells does not occur (data not shown). Thus, despite the high plasma levels of iC3b, it is unlikely that the complement detected on circulating T cells during IL-2 therapy is due to passive adsorption of preformed fragments from the plasma.

In patients with paroxysmal nocturnal hemoglobinuria (PNH), complement activation occurs at the surface of red blood cells due to a deficiency in DAF and homologous restriction factor (HRF), two of several complement regulatory proteins expressed on PB cells.17 We considered the possibility that the ability of IL-2-activated T cells to bind C3 might be due to an acquired deficiency of DAF. Therefore, we measured the expression of this convertase inhibitor on PBMC before and after 5 days of IL-2 therapy cytofluorographically and found no change in the amount or distribution of this protein. These negative results of course do not preclude the possibility that other complement regulatory proteins might disappear from the plasma membrane during IL-2 therapy, thereby facilitating complement activation on the cell surface but we are aware of no evidence that this occurs.

It is also possible that C3 and C5 fragmentation in the plasma of IL-2 recipients is a result of the action of noncomplement enzymes, such as elastase, that are not inhibited by DAF.11,12 In support of this hypothesis, Ramos et al13 and Klein et al14 have shown that T cells stimulated in vitro with lectin or alloantigen activate the alternative pathway of complement by binding C3 fragments generated by T-cell-derived nonconvertase enzymes, a mechanism that may circumvent the inhibitory activities of DAF. The presence of C3 fragments on the surface of activated CD3(+) cells could potentially lead to the binding of factor B and to alternative pathway activation as evinced by the high plasma levels of Ba and Bb (Fig 1).

The elevated plasma levels of C4d in patients receiving IL-2 indicate that the classical pathway is also activated. The hepatic acute-phase reactant CRP is present in high concentrations in the plasma of patients undergoing immunotherapy with IL-217 and has been shown to lead to the fixation of C1 and C4 when complexed to cell surfaces.18 Although receptors for CRP have only been shown on monocytes,19 we have noted that a substantial percentage of CD3(+) lymphocytes from patients receiving IL-2 were reactive with the anti-CRP MoAb, suggesting that CRP was bound to the T-cell surface (Fig 5) and therefore potentially involved in IL-2–induced complement activation. This potential mechanism is analogous to that recently proposed for the development of ARDS in critically ill burn patients receiving parenteral lipid nutritional supplements.20 In these patients, all of whom have high CRP levels, the binding of CRP to lipid droplets and the subsequent activation of the classical complement pathway is thought to be responsible for the ARDS that often complicates the use of these supplements. When PBMC stimulated in vitro with IL-2 were incubated with serum and CRP, the percent of CD3(+) cells binding both the anti-C3c and anti-CRP

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**Fig 7.** Effect of CRP on C3c binding by IL-2-stimulated PBMC in vitro. PBMC from healthy donors were cultured in 1,000 U/mL of IL-2 for 48 hours. The nonadherent cells were then incubated for 50 minutes at 4°C with 30% serum and various concentrations of CRP in GVBS. The cells were then reacted with an anti-C3c MoAb followed by a FITC-conjugated antimouse IgG and a phycoerythrin-conjugated anti-Leu 4 MoAb. The percent of CD3(+) cells reactive with the anti-C3c MoAb was determined by cytofluorography.
MoAbs increased as a function of the CRP concentration (Fig 7). These results indicate that CRP augments the fixation of complement by CD3(+) cells activated in vitro and suggest that during IL-2 therapy (when serum CRP levels are elevated) activated cells bind CRP leading to C4 fixation and the subsequent generation of the fluid-phase C4d detected in the plasma (Fig 1).

The mechanisms by which IL-2-activated CD3(+) cells fix complement and the interaction of these cells with tissues expressing complement receptors are currently under investigation. The studies reported here have indicated that CD3(+) cells activated in vivo bind C3 cleavage products and the terminal complement complex. Yet, despite the presence of the terminal attack unit, these cells are not necessarily lysed as the SC5b-9(+) cells are not excluded as nonviable by the cyttofluorograph. Although the lytic activity of the terminal complex appears to have been abrogated, presumably by membrane-associated regulatory proteins, considerable evidence suggests that this complex may not be entirely inert and may affect the activity of the cells to which it is bound. For example, the sublytic complex SC5b-9 has been shown to activate protein kinase C on tumor cells and could conceivably have similar effects on cells of the immune system. The nonlytic unit SC5b-9 binds to receptors for the S protein (vitronectin) that are present on endothelial and melanoma cells, suggesting that the SC5b-9 structure could serve to target lymphocytes to vitronectin receptor(+) cells and thereby contribute to endothelial injury (capillary leak) and the eradication of tumor cells.

In conclusion, we have found that the administration of high-dose IL-2 induces systemic complement activation and that during the course of IL-2 therapy, CD3(+) lymphocytes acquire the ability to fix C3 and bind CRP. This hepatic acute-phase reactant is present in high concentrations in the serum and appears to augment the ability of IL-2-activated PBMC to fix C3 in vitro and may play a similar enhancing role in vivo. Although the physiologic significance of these derangements remains to be elucidated, we and others strongly suspect that the high levels of circulating anaphylatoxins detected in these patients may contribute to the capillary leak syndrome associated with IL-2 therapy. C5a has been shown to act synergistically with tumor necrosis factor (TNF) to promote inflammation. Because the administration of IL-2 results in the release of TNF into the circulation, it is possible that the effects of the anaphylatoxins C3a and C5a on vascular permeability are similarly amplified by TNF in IL-2 recipients. Potential interactions between these components and other IL-2-induced factors must be considered in the development of strategies to counter the adverse side effects of IL-2 therapy.

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Complement activation in cancer patients undergoing immunotherapy with interleukin-2 (IL-2): binding of complement and C-reactive protein by IL-2-activated lymphocytes

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