Autoreactivity Against Interleukin 6 as a Risk Factor in Elderly Patients With Colorectal Carcinoma

Reiko Wakuda, MD; Chikao Miki, MD, PhD; Masato Kusunoki, MD, PhD

**Hypothesis:** Aging results in both decreased immunity to exogenous antigens and increased autoreactivity. We suggest that the increased autoreactivity against tumor-releasing cachectic cytokine and postsurgical hypercytokinemia are involved in the cause of increased morbidity and mortality in elderly patients with colorectal cancer.

**Design, Setting, and Patients:** Eighty-three patients with colorectal cancer admitted to a university hospital were studied prospectively.

**Interventions:** Surgical specimens of primary colorectal cancer were harvested and peripheral venous blood samples were obtained perioperatively.

**Main Outcome Measures:** The tissue concentrations of interleukin (IL) 1β and IL-6 were determined. Serial determinations of serum concentrations of IL-6, IL-6 soluble receptor, and C-reactive protein were performed. Nutritional status was assessed by the creatinine height index.

**Results:** The tumor IL-6 content was the independent factor that influenced the creatinine height index in the elderly patients, whereas Dukes classification was the only independent factor that influenced the creatinine height index in the younger patients. The elderly patients showed an exaggerated C-reactive protein response and increased IL-6 soluble receptor consumption independent of the tumor IL-6 content and postoperative IL-6 response. This immunologic disturbance was followed by a significant \((P=.03)\) delay in the normalization of activated neutrophils, which seemed to be associated with postoperative fatal complications in the elderly patients.

**Conclusions:** Autoreactivity against intrinsic IL-6 was increased and seemed to be associated with poor clinical outcomes in elderly patients. To prevent fatal complications, adequate nutritional support early in treatment and attenuation of the neutrophil-related hyperinflammatory sequence by controlling the IL-6 soluble receptor affinity should be advocated.

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Improved medical care has increased life expectancy and led to the frequent referral of elderly patients for operation. However, operative stress can provoke major complications, and such complications cause more deaths among elderly patients, suggesting that poor physiologic adaptability in these patients may limit their ability to compensate for postoperative organ failure.

Nutritional depletion is prevalent and continues to be a major cause of morbidity and mortality among surgical patients with gastrointestinal tract malignant neoplasms. Nutritional problems have been increasingly important among elderly patients, since these patients are not able to meet the increased metabolic demand. Clinically, hypermetabolism-associated malnutrition, a common feature in patients with cancer, is related to the presence of a systemic inflammatory response. The role of endogenous mediators of this inflammatory response has not yet been clearly elucidated, although enhanced generation of proinflammatory cytokines is assumed to be involved.

Proinflammatory cytokines have another regulating role in mediating cellular and physiologic inflammatory response after surgical insult. Prolongation of inflammatory response, poorer local defense reactions, and sluggish reparative processes in elderly subjects have been assumed to be involved in the cause of increased morbidity and mortality. Although aging is correlated with a plethora of immunologic changes, resulting in both decreased immunity to exogenous antigens and increased autoreactivity, age-related changes in the human inflammatory response have been largely ignored, and their relationship to clinical outcomes has not been well understood.
PATIENTS AND METHODS

A total of 83 patients who underwent surgery for colorectal cancer were enrolled in the study. Fifty-five of the patients were male. The mean age was 64.7 years (range, 27-86 years). None of these patients had abnormal results of liver function tests or had received nutritional support before surgery. The primary lesion was located in the rectum in 33 patients, the sigmoid colon in 23, the descending colon in 5, the transverse colon in 6, the ascending colon in 11, and the cecum in 5. Seventeen patients were diagnosed as having synchronous liver metastasis. Tumor resection was performed in all patients. The clinicopathologic variables studied for prognostic value were age, sex distribution, tumor size, and Dukes classification. Twenty-one sex- and age-matched healthy subjects were used as control subjects.

For the assessment of protein-energy malnutrition, the creatinine height index (CHI) was measured. The CHI, an index of lean body mass, is defined as the urinary creatinine excretion during a period of 24 hours expressed as a percentage of the value expected to be excreted in healthy subjects of the same height and sex. Fresh surgical specimens of primary colorectal cancer at the edge of the protruded lesion were harvested under sterile conditions from 80 patients. These specimens were immediately placed in liquid nitrogen and stored at −80°C until assay. The samples were thawed, quickly weighed, placed in 5 mL of phosphate-buffered saline, and homogenized for 5 minutes in a tissue homogenizer. The homogenates were then centrifuged twice at 4°C at 12000 rpm, and aliquots of the supernatants were used to measure concentrations of cytokines and protein with enzyme-linked immunosorbent assay kits (interleukin [IL] 1β and IL-6; Endogen, Woburn, Mass) and a protein assay kit (BCA Protein Assay Reagent Kit; Pierce Chemical Company, Rockford, Ill).

Peripheral venous blood samples were obtained on admission, soon after surgery, and on postoperative days 1, 3, and 7. For the assessment of the apoptotic rate of neutrophils, the numbers of circulating lymphocytes and neutrophils were counted and the changes in the percentage of neutrophils in white blood cells against the preoperative level were assessed as described by Kobayashi and Yamauchi. After the numbers of circulating lymphocytes and neutrophils were counted, the blood was allowed to clot, and the serum was separated by centrifugation and was stored at −80°C until assay. The serum concentrations of IL-6 and IL-6 soluble receptor (IL-6sR) were determined with enzyme-linked immunosorbent assay kits (Endogen and R&D Systems, Minneapolis, Minn). The serum level of C-reactive protein (CRP) was measured by a turbidimetric immunoassay (N-Assay TIA CRP-S kit; Nittoho Medical Co., Tokyo, Japan).

Informed consent was obtained from each subject. The results are presented as mean±SD and were subjected to nonparametric Mann-Whitney test or analysis of variance with Bonferroni correction when appropriate. Correlations were analyzed by the Spearman coefficient analysis. Contingency tables were analyzed by Fisher exact test. P<.05 was considered significant. Variables found to be significant at a level of P<.05 were considered eligible for multivariate regression. Data are given as mean±SD unless otherwise stated.

RESULTS

The tumor content of IL-1β ranged from 6.3 to 1745.6 pg/mg of protein, with a mean level of 196.5±40.4 pg/mg of protein. The tumor content of IL-6 ranged from 2.4 to 547.4 pg/mg of protein, with a mean level of 67.0±9.5 pg/mg of protein. The CHI in the patients (range, 24.2-112.7; mean, 73.6) was significantly lower than that in the control subjects (mean, 89.1; P=.04 by Mann-Whitney test) and was negatively correlated with tumor IL-6 content (Figure 1). The CHI in the patients was also negatively correlated with the patient’s age (r = −0.29, P=.04) and associated with the disease progression (Dukes A, 70.8±4.3; B, 83.1±4.9; C, 75.9±3.2; D, 62.6±6.2; B vs D, P<.01 by analysis of variance with Bonferroni correction). However, no other factors, including sex (P=.53), tumor interleukin 1β content (P=.38), and tumor size (P=.38), were associated with the CHI.

The patients were divided into 2 groups according to their ages. The median age of the patients was 65 years. The elderly patient group (mean age, 72 years) was composed of 43 patients who were older than 65 years. The younger patient group (mean age, 55 years) was composed of 40 patients who were younger than 65 years. Multivariate regression models of each patient group showed that the tumor IL-6 content was the independent factor that influenced the CHI in the elderly patient group. Although the association did not reach a statistically significant level, the patient’s age also tended to be associated independently with the CHI in the elderly patient group. In contrast, Dukes classification was the only independent factor that influenced the CHI in the younger patient group (Table 1). The serum concentration of CRP was also correlated with the IL-6 con-

![Figure 1](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3321561/figure/1.png)
centration in the tumors in both patient groups (Figure 2). Although there was no significant difference in the tumor IL-6 concentrations between the 2 patient groups (58.9±14.9 vs 74.5±12.0 pg/mg of protein; \( P = .42 \)), the preoperative circulating level of CRP in the elderly patient group was significantly higher than that in the younger patient group (0.2±0.1 vs 1.4±0.4 mg/dL; \( P = .01 \)).

Table 2 demonstrates the clinical backgrounds of each patient group. There was no significant difference in the disease progression between the 2 groups. Although there were no significant differences in the operative blood loss and operation time between the 2 patient groups, the elderly patients had a significantly higher rate of infectious complications. In addition, all patients with complications in the younger group survived, whereas 2 of 15 patients with complications in the elderly group died because of multiorgan failure. In the elderly group, the patients with infectious complications had a significantly lower level of CHI and a longer operation time. However, in the younger group, the patients with infectious complications had only a higher level of operative blood loss (Table 3).

Figure 3 demonstrates the postoperative serial changes in the serum concentrations of IL-6 and CRP in both patient groups. The serum concentrations of IL-6 increased soon after surgery, then declined continuously until postoperative day 7. The serum concentration of CRP increased after surgery, peaking at postoperative day 3, and then declined until postoperative day 7. Although there was no significant difference in the serum concentrations of IL-6 between the 2 groups, the serum concentration of CRP in the elderly patient group was significantly higher than that of the younger patients during the course of the study.

Figure 4 demonstrates the postoperative survival curves of peripheral neutrophils in the 2 patient groups with or without septic complications. After surgery, the percentage of neutrophils increased rapidly up to 150% of the original value on admission, and then decreased gradually until postoperative day 7. In the patients without septic complications, significant differences in the percentages of neutrophils were found between the 2 groups at postoperative day 7. In the patients with septic complications, significant differences in the percentages of neutrophils were found between the 2 groups at postoperative days 1, 3, and 7.

Figure 5 demonstrates the serial changes in the serum concentration of IL-6sR in the 2 patient groups with or without septic complications. The serum concentration of IL-6sR increased soon after surgery, then declined continuously until postoperative day 7. The serum concentration of IL-6sR in the elderly patient group was significantly lower than that in the younger patient group (0.2±0.1 vs 1.4±0.4 mg/dL; \( P = .01 \)).

Table 1. Multivariate Analysis of Factors Influencing Creatinine Height Index of Younger and Elderly Patients

<table>
<thead>
<tr>
<th>Variables</th>
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<th>( P )</th>
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<tbody>
<tr>
<td>Younger patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.62</td>
<td>.54</td>
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<td>Dukes classification*</td>
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<tr>
<td>Tumor interleukin 6 content</td>
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<tr>
<td>Elderly patients</td>
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<tr>
<td>Age</td>
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<tr>
<td>Dukes classification*</td>
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<td>.80</td>
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<tr>
<td>Tumor interleukin 6 content</td>
<td>-2.30</td>
<td>.03</td>
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*Dukes classification is identical to TNM staging.

Table 2. Clinical Backgrounds of Elderly and Younger Patients

<table>
<thead>
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<th>Variables</th>
<th>Elderly Patients</th>
<th>Younger Patients</th>
<th>( P )</th>
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</thead>
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<tr>
<td>Sex, No. of patients</td>
<td>M 31</td>
<td>F 12</td>
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</tr>
<tr>
<td>Dukes classification†</td>
<td>A 13</td>
<td>B 10</td>
<td>.66</td>
</tr>
<tr>
<td>No. of patients</td>
<td>C 10</td>
<td>D 10</td>
<td>.7</td>
</tr>
<tr>
<td>Tumor size, cm</td>
<td>4.6±0.3</td>
<td>4.5±0.3</td>
<td>.25</td>
</tr>
<tr>
<td>Carcinoembryonic antigen, ng/mL</td>
<td>18.5±8.0</td>
<td>42.1±25.2</td>
<td>.36</td>
</tr>
<tr>
<td>Operative blood loss, mL</td>
<td>579±90</td>
<td>670±90</td>
<td>.49</td>
</tr>
<tr>
<td>Duration of operation, min</td>
<td>314±21</td>
<td>297±21</td>
<td>.57</td>
</tr>
<tr>
<td>Infectious complications, No. of patients</td>
<td>15</td>
<td>6</td>
<td>.046</td>
</tr>
</tbody>
</table>

*Data are given as mean ± SD unless otherwise indicated.†Dukes classification is identical to TNM staging.

Figure 2. Correlation between tumor interleukin 6 content and serum level of C-reactive protein in younger patients (A) and elderly patients (B).
centration of IL-6sR inversely changed with IL-6 in both patient groups. In the patients without septic complications, there was no significant difference in the serum IL-6sR concentration between the elderly and the younger patients. However, the serum concentration of IL-6sR in the elderly patients was significantly lower than that in the younger patients soon after surgery and at postoperative day 1 when they had infectious complications.

**Table 3. Factors Associated With Septic Complications in Elderly and Younger Patients**

<table>
<thead>
<tr>
<th></th>
<th>Blood Loss, mL</th>
<th>Duration of Operation, min</th>
<th>Creatinine Height Index</th>
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</thead>
<tbody>
<tr>
<td><strong>Elderly patients</strong></td>
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<td></td>
</tr>
<tr>
<td>Infectious complications</td>
<td>770 ± 205</td>
<td>365 ± 46</td>
<td>60.8 ± 4.7</td>
</tr>
<tr>
<td>No infectious complications</td>
<td>477 ± 80</td>
<td>286 ± 19</td>
<td>74.6 ± 3.9</td>
</tr>
<tr>
<td><em>P value</em></td>
<td>.12</td>
<td>.07</td>
<td>.04</td>
</tr>
<tr>
<td><strong>Younger patients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infectious complications</td>
<td>1207 ± 276</td>
<td>373 ± 49</td>
<td>90.1 ± 8.1</td>
</tr>
<tr>
<td>No infectious complications</td>
<td>573 ± 94</td>
<td>283 ± 22</td>
<td>75.9 ± 3.5</td>
</tr>
<tr>
<td><em>P value</em></td>
<td>.02</td>
<td>.12</td>
<td>.12</td>
</tr>
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</table>

*Data are given as mean ± SD unless otherwise stated.

Interleukin 6 has a variety of biological effects, mainly on the immune system, in which IL-6 induces the production of various growth factors for tissue regeneration. Interleukin 6 also initiates an integrated fuel substrate adjustment to trauma and sepsis, and helps to provide optimal metabolic homeostasis for systemic host defenses. Although regulation of the IL-6 network is necessary to maintain the host’s biological homeostasis, overproduction of IL-6 often promotes the recruitment of neutrophils to endothelial cells and in some cases may directly damage the tissue parenchyma.

Considerable controversy exists as to the association between aging and cytokine production. Gon et al demonstrated that the stimulated peripheral-blood monocytes of younger patients produced more proinflammatory cytokines compared with those of elderly patients, while increases in proinflammatory cytokine synthesis in the serum or monocytes of elderly subjects have been demonstrated. Ito and Miki recently showed that deterioration of the systemic anti-inflammatory response in patients with colorectal cancer is associated with aging and tumor-related malnutrition.

The present study demonstrated that, although the CRP production increased in association with the tu-

![Figure 3](image-url) Serial changes in serum concentrations of interleukin 6 (IL-6) (A) and C-reactive protein (B) in the 2 patient groups. POD indicates postoperative day.

![Figure 4](image-url) Serial changes in percentages of neutrophils in the 2 patient groups without (A) and with (B) infectious complications. POD indicates postoperative day.
mor IL-6 content in both patient groups, the tumors producing IL-6 caused protein catabolism and were associated with morbidity only in the elderly patients but not in the younger patients. In addition, the postoperative IL-6 response caused a more exaggerated CRP response in elderly patients. Since CRP is an acute-phase reactant that is synthesized in the hepatocytes and is up-regulated by IL-6, IL-6-related metabolic and immunologic disturbances may be exaggerated in elderly patients but are compensated for in younger patients.

Hypercytokinemia is known to prime neutrophils and accumulate primed neutrophils in vital organs. Interleukin 6 also delays neutrophil apoptosis and lymphocytes recovery, thereby inhibiting the resolution of inflammation that may cause secondary organ dysfunction. Moreover, this IL-6-related neutrophil apoptosis is thought to be neutrophil concentration dependent. In the present study, neutrophil apoptosis was significantly delayed in elderly patients, even after the resolution of the postoperative cytokine storm. Moreover, the development of septic complications caused a further delay of apoptosis in these patients. Sepsis-induced necrosis of neutrophils is accompanied by exocytosis of cytotoxic proteases and reactive oxygen metabolites, which promote further tissue inflammation and injury. In contrast, apoptosis of neutrophils is a mechanism of noninflammatory disposal of neutrophils. Since a large population of surviving neutrophils also has a greater collective capacity for superoxide production, the present findings suggest that increased autoreactivity against IL-6 in the elderly patients provokes a delay of neutrophil apoptosis and induces the overproduction of cytotoxic products in neutrophils, which may lead to further tissue injury and thus fatal complications.

Interleukin 6 exerts its action on target cells by acting through a receptor complex consisting of IL-6sR, a specific IL-6 binding protein, and a signal-transducing subunit (gp130). The IL-6–IL-6sR complexes play a positive role in local inflammatory reactions by inducing chemokines. Therefore, IL-6sR may enhance IL-6 activity in cells and reinforce the systemic effects of IL-6. A recent study has shown that, in patients with fatal sepsis, the peak IL-6 level is significantly higher than that in survivors, whereas minimum sIL-6R levels are markedly lower. In surgical patients, IL-6sR is constantly produced in areas other than the operative field, while sIL-6R level is reduced by consumption in the operative field. In the present study, the consumption of IL-6sR was highly exaggerated in the elderly patients when they had septic complications, suggesting that sepsis provokes the consumption of IL-6sR, which systemically enhances the IL-6 activity and leads to fatal disease. These findings indicate that the increased affinity of IL-6 with IL-6sR in the elderly patients may partly explain their increased autoreactivity against IL-6.

In conclusion, autoreactivity against IL-6 was increased and seems to be associated with poor clinical outcomes in elderly patients with colorectal carcinoma. Postoperative IL-6 response is known to reflect surgical stress and predict the development of complications in surgical patients. However, the autoreactivity against IL-6 may be a more important factor that was associated with clinical outcomes in elderly patients. Aging itself may be a form of stress, and inflammatory responses may increase as a function of normal aging and be correlated inversely with levels of functional ability in elderly people. The risk factor mentioned herein is particularly important because some pathologic states can be corrected, and some other preventive measures can be taken before surgery, as long as one is not dealing with an emergency. Since serum CRP seems to be a useful indicator of IL-6-induced metabolic and immunologic disturbances in elderly patients, the challenge is to identify these vulnerable patients and to prevent fatal organ failure by providing adequate nutritional support early in treatment and attenuating the neutrophil-related hyperinflammatory sequence by controlling the IL-6sR affinity.

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REFERENCES


