High Innate Production Capacity of Proinflammatory Cytokines Increases Risk for Death from Cancer: Results of the PROSPER Study

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Abstract

Purpose: Various lines of evidence suggest that proinflammatory factors may play a role in tumor growth and metastasis, the leading cause of cancer-related mortality. However, most evidence originates from animal models, only few human studies reported an association between proinflammatory cytokines and death from cancer. Here, we investigated the association between circulating levels and innate production capacity of proinflammatory cytokines and cancer incidence and mortality in the Prospective Study on Pravastatin in the Elderly at Risk (PROSPER).

Experimental Design: Circulating levels of interleukin 6 (IL-6) and C-reactive protein were measured in all 5,804 participants of the PROSPER study. The innate production capacity of IL-6, IL-1β, and tumor necrosis factor α (TNF-α) were measured in a random sample of 403 subjects.

Results: We showed that high circulating inflammatory markers were associated with an increased risk for cancer incidence and death from cancer during follow-up (all \( P < 0.05 \)). Moreover, high innate proinflammatory cytokine production capacity was associated with an increased risk for death from cancer (all \( P < 0.04 \)) but not with higher cancer incidence during follow-up (all \( P > 0.6 \)).

Conclusions: High innate production capacity of proinflammatory cytokines is associated with an increased risk for death from cancer, probably because of increased tumor growth and metastasis. Because there was no association between innate production capacity and cancer incidence, the association between circulating levels and cancer incidence at least partially reflects reversed causality. (Clin Cancer Res 2009;15(24):7744–8)

Inflammation plays an important role in the development of various age-related diseases such as atherosclerosis, stroke, cognitive decline, and dementia (1). Various studies support the hypothesis that inflammatory stimuli, such as the proinflammatory cytokines interleukin 6 (IL-6), IL-1β, and tumor necrosis factor α (TNF-α), are involved in cancer pathogenesis (2–5). Moreover, elevated levels of various cytokines, such as IL-1, IL-6, TNF, fibroblast growth factor, and transforming growth factor, have been found in blood, urine, and ascites of cancer patients, suggesting that these cytokines are involved in incidence and growth and spread of cancer (6).

Inflammatory responses are thought to be critical in many aspects of promoting the growth and spread of cancers. A recent study on Kim et al. (7) showed that cell lines of Lewis lung carcinoma had an increased production of the proinflammatory cytokines IL-6 and TNF-α through activation of the Toll-like receptor family members TLR2 and TLR6. Moreover, proinflammatory cytokines are also involved in promoting tumor cell adhesion in metastatic sites, which then activate local normal cells to produce tumor growth factors (6). Distant-site metastases are the leading cause of cancer-associated mortality. Furthermore, animal studies have suggested a role for proinflammatory cytokines in the generation of cancer-associated cachexia, which

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We assessed the association between circulating levels and innate production capacity of proinflammatory cytokines in whole blood samples and cancer incidence and mortality. High innate production capacity of proinflammatory cytokines is associated with an increased risk for cancer mortality, probably because of increased tumor growth and metastasis. No association was found between innate production capacity and cancer incidence, which indicates that the association between circulating levels and cancer incidence is probably disturbed by reversed causality. Anticytokine therapy for the interleukin 1β, interleukin 6, and tumor necrosis factor α cytokines might be of therapeutic interest for advanced cancer. Blocking the proinflammatory cytokines by anticytokine-based therapies might reduce tumor growth and metastasis, the leading cause of cancer-associated mortality. Moreover, it might reverse cachexia-induced weight loss. Hence, when tumor growth and progression and cancer-related cachexia can be delayed or reversed by administering antibodies against proinflammatory cytokines, the survival time for cancer patients might be extended.

**Materials and Methods**

A detailed description of the protocol of the PROSPER study has been published elsewhere (13, 14). A short summary is provided here.

**Participants.** PROSPER was a prospective multicenter randomized placebo-controlled trial to assess whether treatment with pravastatin diminishes the risk for major vascular events in the elderly. Between December 1997 and May 1999, we screened and enrolled subjects in Scotland (Glasgow), Ireland (Cork), and the Netherlands (Leiden). Men and women with ages 70 to 82 y were recruited if they had pre-existing vascular disease or increased risk for such disease because of smoking, hypertension, or diabetes. A total number of 5,804 subjects were randomly assigned to pravastatin or placebo.

**Inflammatory markers.** In all subjects, C-reactive protein (CRP) was measured on stored (at -80°C) and previously unthawed samples by automated particle-enhanced immunoturbidimetric assay (Roche UK). The method has interassay and intra-assay coefficients of variation of <3%. The laboratory participates in the United Kingdom national external quality control for high-sensitivity CRP. IL-6 was assayed using a high-sensitivity enzyme-linked immunosorbent assay (R and D Systems) with interassay and intra-assay coefficients of variation of <6%. All samples were processed by technicians blinded to the identity of samples.

Innate cytokine production capacity was measured in the final 30% of the Dutch participants at baseline and resulted in a random subsample of 403 subjects. Whole blood samples were stimulated with 10 ng/mL of lipopolysaccharide to assess the innate production capacity of IL-1β, IL-6, and TNF-α. Unstimulated baseline samples were obtained to serve as a control for contamination.

**Cancer incidence and mortality.** All subjects included into the PROSPER study did not have a history of malignancy within the 5 y before the start of the trial. Tertiary study endpoints of the PROSPER trial included cancer incidence and mortality. All study endpoints were adjudicated by a study endpoint committee. We extended the follow-up period for the 1,100 Dutch participants of the PROSPER study. First, incident cancer was requested for the 1,100 subjects at the Dutch cancer registry for the period December 1997 and May 1999 until September 2005 (censor date was 15 September 2005). For the same period, the mortality status was checked for the 1,100 Dutch participants. From the deceased participants, the cause of death was obtained from the Dutch Central Bureau of Statistics. Only the primary cause of death on the death certificate was taken into account.

**Statistical analysis.** The circulating CRP and IL-6 measurements of all subjects and the innate cytokine production levels of the 403 participants were dichotomized in two groups based on the median cytokine production level. All associations between the two groups of cytokine production levels and cancer incidence or death from cancer were assessed with a Cox proportional hazard model adjusted for sex, age, current smoking, use of pravastatin, and country, where appropriate. These associations were visually depicted with Kaplan-Meier survival curves. The SPSS software (version 16.0.1; SPSS, Inc.) was used for all statistical analyses. P < 0.05 was considered statistically significant.

**Results**

Baseline characteristics of the 5,804 subjects of the PROSPER study are presented in Table 1. The mean age of the subjects was 75.3 y, and about half of them were female. The baseline characteristics of the random sample of the 403 subjects with additionally obtained innate cytokine production capacities are also shown in Table 1. Both groups were similar in baseline characteristics. Cancer incidence and mortality were measured.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Total group (N = 5,804)</th>
<th>Random sample (n = 403)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>75.3 (3.3)</td>
<td>75.1 (3.3)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>3,000 (52)</td>
<td>187 (46)</td>
</tr>
<tr>
<td>Education</td>
<td>15.1 (2.0)</td>
<td>15.0 (2.8)</td>
</tr>
<tr>
<td>Current smokers, n (%)</td>
<td>1,558 (27)</td>
<td>96 (24)</td>
</tr>
<tr>
<td>Weight</td>
<td>73.4 (13.4)</td>
<td>77.7 (11.6)</td>
</tr>
<tr>
<td>Body mass index</td>
<td>26.8 (4.2)</td>
<td>26.9 (3.6)</td>
</tr>
<tr>
<td>Cancer, n (%)</td>
<td>444 (8)*</td>
<td>45 (11)*</td>
</tr>
<tr>
<td>Cancer incidence</td>
<td>206 (4)*</td>
<td>26 (7)</td>
</tr>
</tbody>
</table>

NOTE: Data is presented as mean (SD) unless otherwise stated. *Measured after 3 y of follow-up. †Measured after 7 y of follow-up.

PROSPER was a prospective multicenter randomized placebo-controlled trial to assess whether treatment with pravastatin diminishes the risk for major vascular events in the elderly. Between December 1997 and May 1999, we screened and enrolled subjects in Scotland (Glasgow), Ireland (Cork), and the Netherlands (Leiden). Men and women with ages 70 to 82 y were recruited if they had pre-existing vascular disease or increased risk for such disease because of smoking, hypertension, or diabetes. A total number of 5,804 subjects were randomly assigned to pravastatin or placebo.
for the total group for a mean follow-up period of 3.2 years; for the random sample, we extended the initial follow-up period with 3.5 to 6.7 years. The percentages of cancer incidence and cancer mortality are therefore higher in the random sample.

The association between circulating inflammatory markers and cancer risk is shown in Table 2. The hazard ratio for cancer incidence for subjects with high levels of CRP was $1.20 (P = 0.063)$ compared with subjects with low CRP levels. Moreover, the hazard ratio for cancer incidence for subjects with high levels of IL-6 was $1.35 (P = 0.003)$ compared with subjects with low IL-6 levels. High levels of both inflammatory markers were also significantly associated with an increased risk for death from cancer compared with low levels [$1.42 (P = 0.01)$ and $1.55 (P = 0.003)$, respectively].

In Table 3, the association is shown between the innate production capacity and cancer risk in a random sample of 403 subjects. No associations were found for innate cytokine production capacity and cancer incidence. However, innate production capacity levels of the proinflammatory cytokines IL-1β, IL-6, and TNF-α were significantly associated with death from cancer (all $P < 0.04$). Participants with high production capacity levels of these cytokines had a higher risk for death from cancer compared with participants with low cytokine production levels. There was no association between high IL-1β and TNF-α cytokine production levels and other causes of death, whereas high IL-6 production capacity was also associated with an increased risk for all other deaths except cancer ($1.92; P = 0.04$).

The Kaplan-Meier curves of the association between the innate production capacity and mortality from cancer are graphically presented in Fig. 1. The Kaplan-Meier curves show the cumulative mortality from cancer for subjects with high (dotted line) and low (straight line) innate cytokine production capacity.
depicted in Fig. 1. Subjects with high levels of IL-1β, IL-6, and TNF-α production capacity had a higher cumulative mortality compared with subjects with low production capacity.

Discussion

We assessed the association between circulating levels and innate production capacity of proinflammatory cytokines in whole blood samples and cancer incidence and mortality. High levels of the circulating inflammatory markers were associated with an increased risk for cancer incidence and death from cancer. Furthermore, we showed that high innate proinflammatory cytokine production capacity was associated with an increased risk for death from cancer during follow-up, whereas high innate production capacity of proinflammatory cytokines was not associated with incident cancer.

We found that a high innate proinflammatory cytokine production capacity is a risk factor for cancer mortality but not for cancer incidence and also not for any other causes of death. This indicates that circulating markers of inflammation are increased in cancer patients, probably by autocrine production of the cancer cells themselves. There are two ways to explain the association between the innate production capacity of IL-1β, IL-6, and TNF-α, and death from cancer. First, proinflammatory cytokines play an important role in promoting the growth and spread of cancers. There are some examples of solid tumors proliferating in response to IL-1, IL-2, and IL-6 (6). Cytokines are also involved in promoting tumor cell adhesion in metastatic sites and then activate local normal cells to produce tumor growth factors (6). Furthermore, TNF-α receptors have been associated with tumor cells, suggesting that TNF-α could play a role in cancer growth (15–17). The most convincing evidence comes from the recent study on Kim et al. (7) who reported that cell lines of Lewis lung carcinoma had an increased production of the proinflammatory cytokines IL-6 and TNF-α through activation of the Toll-like receptor family members TLR2 and TLR6. Moreover, TNF-α and TLR2 were found to be required for Lewis lung carcinoma metastases in mice (7).

Second, animal studies have suggested that proinflammatory cytokines may have a role in cancer related cachexia, which is an important cause of morbidity and mortality in cancer patients (3, 8–10). In a tumor model used by Strassmann et al. (18, 19), it was suggested that IL-1 and IL-6 are involved in mediating cachexia. Administering IL-6 antibodies in a similar model partially reversed the weight loss. Mice with TNF-α producing tumors also developed cachexia, and administration of TNF neutralizing antibodies reversed the weight loss related to cachexia (16).

Although we found an association between circulating inflammatory markers and cancer incidence, we found no association between innate production capacity and incident cancer. This might indicate that the association between circulating inflammatory markers and cancer incidence might be disturbed by reverse causality because it has been shown in various studies that tumor cells have autocrine production of proinflammatory cytokines (6). Although all participants of the PROSPER study had to be free of cancer in the 5 years before the study, underlying cancer that had not been diagnosed yet could have resulted in higher levels of circulating inflammatory markers. Alternatively, strong cancer risk factors may have contributed to an altered inflammatory milieu. By investigating the association between innate cytokine production capacity and cancer incidence, we do not have the problem of reverse causality because innate production capacity reflects the maximum response to lipopolysaccharide in an individual independent of cytokine production by tumor cells. Therefore, we suggest that subjects with cancer a strong proinflammatory profile are associated with an increased risk for dying, but the increased innate production capacity does not lead to an increased risk for developing cancer.

A possible limitation to use the PROSPER study cohort for this research question is that subjects were selected to have a history of vascular disease or have an increased risk for such a disease, and the results can only be extrapolated with this in mind to the general population. One of the strengths of our study is our population size. We had prospective data of >5,000 subjects on various outcomes in three different countries. Because of the large population size, we had sufficient cases of incident cancer to reach a high power for statistical analyses. Furthermore, all subjects were included into the study when they did not have a history of malignancy within the 5 years before the start of the trial. Cancer incidence and mortality were main outcomes of our study and were accurately monitored. In addition, the fact that we had a follow-up of 3.2 years for all subjects with little lost to follow-up is a strong element of our study.

In conclusion, high innate production capacity of proinflammatory cytokines is associated with an increased risk for cancer mortality, probably because of increased tumor growth and metastasis. No association was found between innate production capacity and cancer incidence, which indicates that the association between circulating levels and cancer incidence is probably disturbed by reversed causality. Anticytokine therapy for the IL-1b, IL-6, and TNF-α cytokines might be of therapeutic interest for advanced cancer (20, 21). Blocking the proinflammatory cytokines by anticytokine based therapies might reduce tumor growth and metastasis, the leading cause of cancer-associated mortality. Moreover, it might reverse cachexia-induced weight loss (22). Hence, when tumor growth and progression and cancer-related cachexia can be delayed or reversed by administering antibodies against proinflammatory cytokines, the survival time for cancer patients might be extended.

Disclosure of Potential Conflicts of Interest

All authors declare that they have no conflicts of interest.

References

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