Genes, inflammation, and age-related diseases

Trompet, S.

Chapter 6

High innate production capacity of pro-inflammatory cytokines increases risk of death from cancer. Results of the PROSPER study.

Stella Trompet, Anton JM de Craen, Simon Mooijaart, David J Stott, Ian Ford, Naveed Sattar, J Wouter Jukema, Rudi GJ Westendorp.

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Abstract

Various lines of evidence suggest that pro-inflammatory 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 pro-inflammatory cytokines and death from cancer. Here, we investigated the association between circulating levels and innate production capacity of pro-inflammatory cytokines and cancer incidence and mortality in the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). Circulating levels of IL-6 and CRP were measured in all 5804 participants. The innate production capacity of IL-6, IL1β and TNFα were measured in a random sample of 403 subjects. We showed that high circulating inflammatory markers were associated with an increased risk of cancer incidence and death from cancer during follow-up (all p< 0.05). Moreover, high innate pro-inflammatory cytokine production capacity is associated with an increased risk of death from cancer (all p<0.04) but not with higher cancer incidence during follow-up (all p> 0.6). In conclusion, high innate production capacity of pro-inflammatory cytokines is associated with an increased risk of death from cancer probably due to increased tumor growth and metastasis. As 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.

Introduction

Inflammation plays an important role in the development of various age-related diseases like atherosclerosis, stroke, cognitive decline, and dementia (4). Various studies support the hypothesis that inflammatory stimuli, like the pro-inflammatory cytokines interleukin-6 (IL-6), interleukin-1-beta (IL-1β), and tumor necrosis factor-alpha (TNFα) are involved in cancer pathogenesis (5-8). Moreover, elevated levels of various cytokines, like IL-1, IL-6, TNF, fibroblast growth factor (FGF), and transforming growth factor (TGF) 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 (9).

Inflammatory responses are thought to be critical in many aspects of promoting the growth and spread of cancers. A recent study of Kim *et al* showed that cell lines of Lewis lung carcinoma had an increased production of the pro-inflammatory cytokines IL-6 and TNFα through activation of the Toll-like receptor (TLR) family members TLR2 and TLR6 (10). Moreover, pro-inflammatory cytokines are also involved in promoting tumor cell adhesion in metastatic sites which then activate local normal cells to produce tumor growth factors (9). Distant-site metastases are the leading cause of cancer-associated mortality. Furthermore, animal studies have suggested a role for pro-inflammatory cytokines in the generation of cancer-associated cachexia, which is the most important cause of morbidity among cancer patients (6;11-13).

These various lines of evidence suggest that pro-inflammatory factors may play a role in cancer metastasis eventually leading to death. However, most evidence originates from animal models, only a few human studies have reported an association between pro-inflammatory cytokines and death from cancer (14;15). Here, we investigated the association between circulating levels and innate production capacity of pro-inflammatory cytokines and cancer incidence and mortality in the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER).

Methods

A detailed description of the protocol of the PROSPER Study has been published elsewhere (16;17). 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 of 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 aged 70-82 years were recruited if they had pre-existing vascular disease or increased risk of such disease because of smoking, hypertension, or diabetes. A total number of 5804 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, Welwyn Garden City, UK). The method has inter- and intra-assay coefficients of variation of <3%. The laboratory participates in the United Kingdom national external quality control for high-sensitivity CRP. Interleukin-6 was assayed using a high-sensitivity ELISA (R & D Systems, Oxford, UK) with inter 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, resulted in a random subsample of 403 subjects. Whole blood samples were stimulated with 10 ng/ml of lipopolysaccharide (LPS) to assess the innate production capacity of IL-1β, IL-6, and TNFα. 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 years prior to 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 1100 Dutch participants of the PROSPER study. First, incident cancer was requested for the 1100 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 1100 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, Chicago, Ill) was used for all statistical analyses. P-values lower than 0.05 were considered statistically significant.

Results

Baseline characteristics of the 5804 subjects of the PROSPER study are presented in table 1. The mean age of the subjects was 75.3 years 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.

Table 1: Baseline characteristics of the participants of the PROSPER study

	Total Group (N = 5804)	Random Sample (N = 403)
Demographics		
Age	75.3 (3.3)	75.1 (3.3)
Female, N(%)	3000 (52)	187 (46)
Education ≥ 13 years	15.1 (2.0)	15.0 (2.8)
Current smokers, N(%)	1558 (27)	96 (24)
Weight	73.4 (13.4)	77.7 (11.6)
Body Mass Index	26.8 (4.2)	26.9 (3.6)
Cancer, N(%)		
Cancer incidence	444 (8)*	45 (11)†
Cancer mortality	206 (4)*	26 (7)†

Data is presented as mean (SD) unless otherwise stated.

* Measured after 3 years of follow-up

† Measured after 7 years of follow-up

Cancer incidence and mortality was measured 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 years to 6.7 years.

The percentages of cancer incidence and cancer mortality are therefore higher in the random sample.

Table 2: Association between circulating levels of inflammatory markers and cancer risk

	Inflammatory marker			
	< Median n/N (%)	> Median n/N (%)	Hazard ratio (95% CI)	p-value
Cancer incidence				
CRP	197/2838 (7)	234/2842 (8)	1.20 (0.99-1.45)	0.063
Interleukin-6	179/2826 (6)	253/2827 (9)	1.35 (1.11-1.64)	**0.003**
Cancer mortality				
CRP	82/2838 (3)	119/2842 (4)	1.42 (1.07-1.89)	**0.014**
Interleukin-6	78/2826 (3)	123/2827 (4)	1.55 (1.16-2.07)	**0.003**

Hazard ratios are assessed with the Cox-proportional hazard model adjusted for sex, age, country, current smokers, and use of pravastatin.

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=0.06) compared to subjects with low CRP levels. Moreover, the hazard ratio for cancer incidence for subjects with high levels of interleukin-6 was 1.35 (p=0.003) compared to 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 to low levels (HR=1.42, p=0.01 and HR=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 pro-inflammatory cytokines IL-1β, IL-6, and TNFα were significantly associated with death from cancer (all p-values below 0.04). Participants with high production capacity levels of these cytokines had a higher risk for death from cancer compared to participants with low cytokine production levels. There was no association between high IL-1β and TNFα 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 (HR 1.92, p=0.04).

Table 3: Association between innate inflammatory cytokine production capacity and cancer risk

	Cytokine level			
	< Median n/N (%)	> Median n/N (%)	Hazard ratio (95% CI)	p-value
Cancer incidence				
IL-1β	24/201 (12)	20/202 (10)	0.85 (0.47-1.55)	0.60
IL-6	21/198 (11)	22/199 (11)	1.11 (0.61-2.02)	0.74
TNFα	23/201 (11)	21/202 (10)	0.92 (0.51-1.67)	0.78
Cancer mortality				
IL-1β	8/201 (4)	18/202 (9)	2.67 (1.15-6.20)	**0.02**
IL-6	8/198 (4)	18/199 (9)	2.51 (1.09-5.81)	**0.03**
TNFα	7/201 (3)	19/202 (9)	3.14 (1.31-7.54)	**0.01**

Hazard ratios are assessed with the Cox-proportional hazard model adjusted for sex, age, current smokers, and use of pravastatin.

Discussion

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

We found that a high innate pro-inflammatory 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 TNFα and death from cancer. First, pro-inflammatory cytokines play an important role in promoting the growth and spread of cancers.

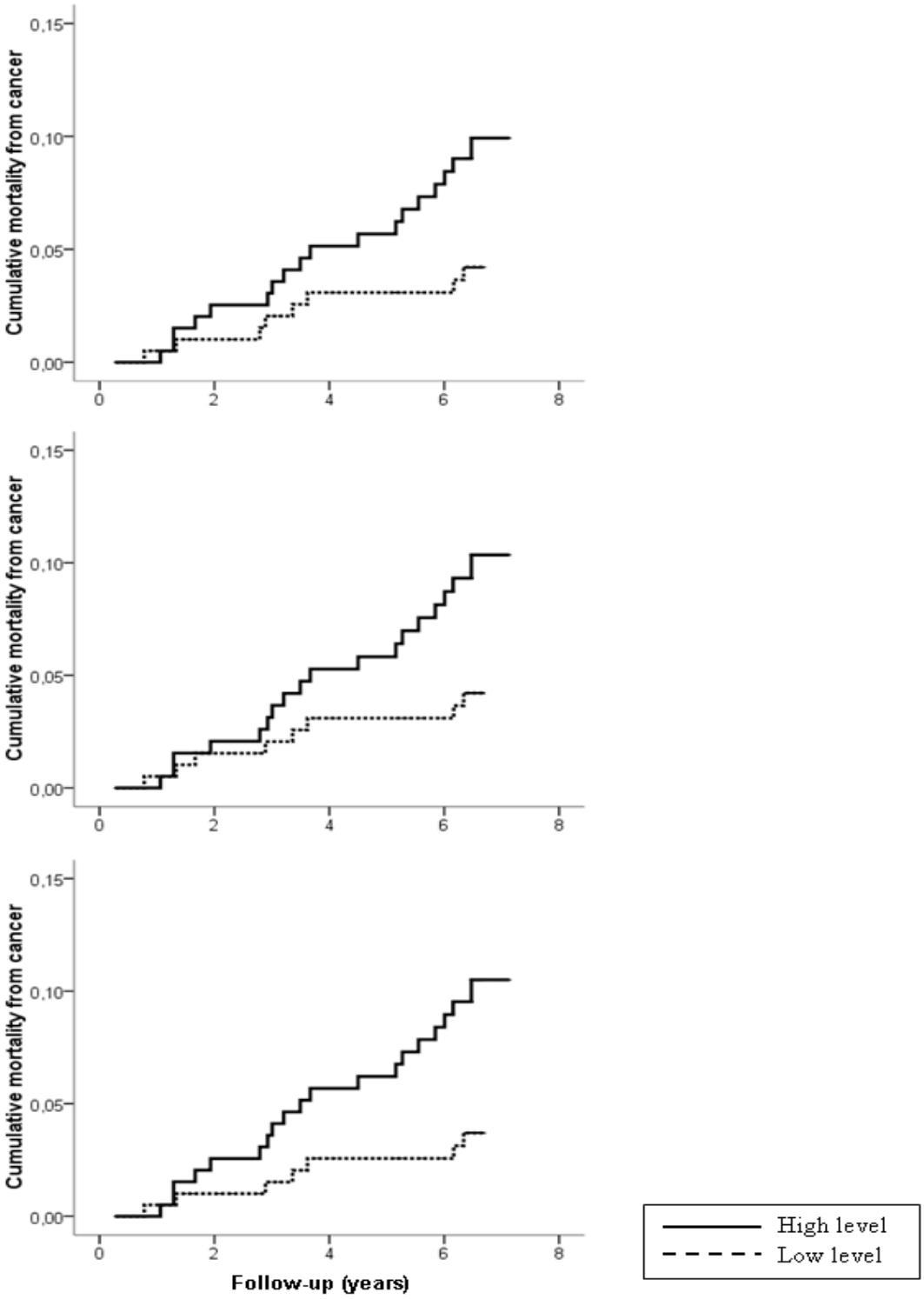

Figure 1: Kaplan-Meier curves

The dotted line indicates high innate cytokine production capacity; the straight line indicates low innate cytokine production capacity.

There are some examples of solid tumors proliferating in response to IL-1, IL-2 and IL-6 (9). Cytokines are also involved in promoting tumor cell adhesion in metastatic sites and then activate local normal cells to produce tumor growth factors (9). Furthermore, TNFα receptors have been associated with tumor cells, suggesting that TNFα could play a role in cancer growth (18-20). The most convincing evidence comes from the recent study of Kim *et al* who reported that cell lines of Lewis lung carcinoma had an increased production of the pro-inflammatory cytokines IL-6 and TNFα through activation of the Toll-like receptor (TLR) family members TLR2 and TLR6 (10). Moreover, both TNFα and TLR2 were found to be required for Lewis lung carcinoma metastases in mice (10).

Second, animal studies have suggested that pro-inflammatory cytokines may have a role in cancer related cachexia, which is an important cause of morbidity and mortality in cancer patients (6;11-13). In a tumor model used by Strassmann *et al* it was suggested that IL-1 and IL-6 are involved in mediating cachexia (21;22). Administrating IL-6 antibodies in a similar model partially reversed the weight loss. Mice with TNFα producing tumors also developed cachexia and administration of TNF neutralizing antibodies reversed the weight loss related to cachexia (19).

While 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 since it has been shown in various studies that tumor cells have autocrine production of pro-inflammatory cytokines (9). Although all participants of the PROSPER study had to be free of cancer in the 5 years prior to the study, underlying cancer which 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, since innate production capacity reflects the maximum response to LPS in an individual independent of cytokine production by tumor cells. Therefore we

suggest that in subjects with cancer a strong pro-inflammatory profile is associated with an increased risk of 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 over 5000 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 prior to the start of the trial. Cancer incidence and mortality were main outcomes of our study and were accurately monitored. Also 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 pro-inflammatory cytokines is associated with an increased risk of cancer mortality probably due to 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. Anti-cytokine therapy for the IL-1b, IL-6, and TNFa cytokines might be of therapeutic interest for advanced cancer (1;2). Blocking the pro-inflammatory cytokines by anti-cytokine based therapies might reduce tumor growth and metastasis, the leading cause of cancer-associated mortality. Moreover it might reverse cachexia-induced weight loss (3). Hence, when tumor growth and progression and cancer-related cachexia can be delayed or reversed by administering antibodies against pro-inflammatory cytokines, the survival time for cancer patients might be extended.

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