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Low innate production of interleukin-1β and interleukin-6 is associated with the absence of osteoarthritis in old age

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SUMMARY

Objective: We investigated whether innate differences in cytokine response were associated with the absence of osteoarthritis (OA) in old age.
Design: In 82 participants from a cross-sectional birth cohort, radiographs of hands, hips and knees were taken at the age of 90 years. OA was defined as a Kellgren–Lawrence score of at least two. "Free from OA" was defined at patient level as absence of hip and knee OA, and presence of OA in maximally two hand joints. The innate cytokine response was determined in whole-blood samples upon stimulation with lipopolysaccharide. Logistic regression analyses were used to investigate associations between absence of OA in relation to tertiles of interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, IL-1 receptor antagonist (RA) and IL-10. Adjustments were made for gender and body mass index.
Results: Sixteen percent of the participants were "free from OA". Subjects in the lowest tertile of Il-1β production had a 11-fold increased chance to be free of OA [odds ratio (OR) 11.3, confidence intervals (CI) 95% 1.1–115.9], subjects in the lowest tertile of IL-6 production had an almost 7-fold increased chance to be free of OA (OR 6.7, 95% CI 1.1–41.2). Absence of hand OA was associated with low innate production of IL-6 and IL-1RA, absence of hip OA was associated with low innate IL-1β production. No associations were found for TNF-α and IL-10.
Conclusions: Low innate capacity to produce the pro-inflammatory cytokines IL-1β and IL-6 is associated with the absence of OA in old age.

Introduction

Osteoarthritis (OA) is the most prevalent joint disease and a frequent cause of musculoskeletal disability in developed countries. It is not regarded as one distinct disease, but rather the result of a complex interplay of predisposing hormonal, metabolic and genetic factors, and local biomechanical factors, accounting for the heterogeneity in OA phenotypes. Although age is generally accepted as the strongest risk factor for OA, the prevalence and incidence of OA decline after the seventh decade[1,2]. Few data are available on the cause of this phenomenon[2,3], but one could hypothesize that factors exist that protect against OA.

Cytokine-driven anabolic and catabolic processes are involved in the integrity of articular cartilage. Pro-inflammatory cytokines have been demonstrated to play a pivotal role in the development of the OA disease process. In particular, interleukin (IL)-1β and tumor necrosis factor (TNF)-α seem to play a prominent role and are of major importance to cartilage destruction and remodeling of the subchondral bone[4–10]. Relatively large interindividual differences exist in the *ex-vivo* production of several cytokines upon the same stimulus. These differences are caused by genetic factors as is shown in twin studies[11–13]. The variation in DNA sequence that explains these differences has not been identified yet. Thus the *ex-vivo* production of cytokines is commonly used as an intermediate phenotype to investigate associations between production of cytokines and diseases such as OA.

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The investigation of factors that protect against OA can most effectively be performed in subjects without OA, moreover, in subjects that would have had a high chance to develop OA. Since OA is a slowly evolving disease, this type of study cannot be performed in subjects of middle age or in subjects in their seventies, but rather in old age, especially using the extreme phenotype of absence of OA at very old age.

Therefore, we tested the hypothesis whether absence of OA at 90 years of age was associated with a low innate *ex-vivo* pro-inflammatory cytokine production or high innate *ex-vivo* anti-inflammatory cytokine production.

Method

Study population

In April 2004 all participants of the Leiden 85-plus Study (age range 89–91) were invited to take part in the present subsidiary study. The Leiden 85-plus Study is a prospective population based follow-up study of all 85-year-old inhabitants of Leiden, The Netherlands. The study design and characteristics of the cohort were described in detail previously[14]. In short, between September 1997 and September 1999 all 705 members of the 1912–1914 birth-cohorts in the city of Leiden were invited to participate in the month after their 85th birthday. The initial study cohort consisted of 599 participants (87% response rate) at age 85 years. There was no selection criterion related to health or demographic characteristics.

All participants gave informed consent to enter the study but for people who were severely cognitively impaired, a guardian gave informed consent. The Medical Ethics Commission of Leiden University approved the study.

Demographic and clinical characteristics

Demographic data and medical history were collected when participants were aged 85 years. At the age of 90 years, a research nurse visited participants at home and collected information by questionnaire on demographic characteristics. The medical history, regarding OA ("history of OA") and co-morbid conditions were obtained from participants' general practitioners or nursing home physicians by standardized interviews. When participants were 90 years their weight and height were measured by the research nurse.

Radiographic examination and assessment of OA

Radiographic OA was chosen as phenotype since the aim of the present study was to investigate etiology and pathogenesis. In participants who were able to visit the hospital radiographs of hands (dorso-volar), hips (anterior–posterior (AP), supine) and knees (weight-bearing, posterior–anterior (PA), full-extension) were obtained according to a standardized method with a fixed film-focus distance and a fixed position of the joint as was shown before (submitted). Cognitive impairment was the major reason not to be able to come to the study center.

In short, radiographs were assessed for OA by two independent readers (HMK and RJG), who were blinded to patient characteristics. The method of scoring OA followed that described by Kellgren and Lawrence (K–L) (score range 0–4)[15]. The following joints were scored: eight distal interphalangal joints (DIPJs), eight proximal interphalangal joints (PIPJs), two first interphalangal joints (first IPJs) and two first carpometocarpal joints (first CMCJs), both hips and both femoro-tibial joints.

The inter-observer reliability for a K–L ≥ 2 as a dichotomous variable expressed by the κ statistic varied from 0.88 for the right hip to 0.41 for the right first IPJs. In cases of disagreement between readers' evaluation of the radiographic OA status of a joint, a rescore was performed to reach consensus, which was used for calculations.

OA diagnosis

OA for each joint was defined as a K–L score ≥ 2. Overall presence of hand OA was defined as a K–L score of ≥ 2 in three or more joints of all scored hand joints. This cutoff was chosen since radiological OA in one or two hand joints could be the result of single traumatic lesions. This paper focuses on systemic pathophysiology of OA, hence single lesions potentially due to trauma alone are considered outside the scope of this paper. A participant was considered "free from OA" when no OA was present in the hips and knees and hand OA was absent. A joint prosthesis in a particular joint was regarded as OA if this was the indication for the operation. If the reason was unknown, the joint was not included in the rating.

Cytokine production capacity of the innate immune system

At the age of 85 and 86 two venous blood samples were drawn. The cytokine production capacity of the innate immune system was assessed by stimulating *ex-vivo* whole-blood samples with lipopolysaccharide (LPS) as described before[16]. Cytokine production by this method is under tight genetic control and production levels have been shown to be stable over time[12,17]. In short, all venous blood samples were drawn in the morning, diluted 2-fold with RPMI-1640, and stimulated with 10 ng/ml *Escherichia coli*-derived LPS (Difco Laboratories, Detroit, MI, USA) before 11.00 am to exclude circadian variation. After 4 h and after 24 h of incubation at 37°C and 5% CO_2, supernatants were collected and stored at −80°C to measure TNF-α and IL-1β, IL-6, IL-10, and IL-1 receptor antagonist (RA), respectively. Standard enzyme-linked immuno sorbent assay (ELISA) techniques were performed according to the manufacturers' guidelines (Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands). Three participants had unstimulated TNF-α production >0.10 pg/ml and were thought to have a temporarily inflammatory condition and were excluded from further analyses. Participants did not use immunosuppressive drugs. Between test agreement was 79% for both low and high producers (lowest and highest tertiles of cytokine production of LPS stimulated IL-1β), overall test agreement was moderate assessed by Cohen's Kappa ($\kappa = 0.51$). The average of the two observations was used for analyses.

Statistical analyses

Differences in demographic and clinical characteristics between participants who were able to visit the study center for radiographic examination vs those who were not, and between participants with OA vs those without OA were determined using two-sided independent Student's *T*-tests for continuous variables and with Chi-square tests for non-continuous variables.

The mean difference in the innate *ex-vivo* production of cytokines between participants with and without OA was calculated by linear mixed models to take into account the two available levels for every participant. A covariance pattern model was used with body mass index (BMI), sex and their interaction in the matrix model. Since the *ex-vivo* production of cytokines was not normally distributed, a log transformation was performed. Estimates of fixed effects were reported with 95% confidence intervals (95% CI). Adjustments were made for gender, BMI and patient-effect. The

estimates represent the magnitude of the difference in the mean innate *ex-vivo* production of cytokines between participants with and without OA.

Subsequently, the association between the tertiles of cytokine levels, based on the distribution in the participants, and absence of OA was determined by logistic regression analysis. Odds ratios (ORs) with 95% CI were calculated, presented within parentheses. Adjustments were made for gender and BMI. All analyses were carried out using statistical analysis software, SPSS version 16.0 (SPSS Inc, Chicago, Ill, USA).

Results

Study population

Two hundred and ninety-one 90-year-olds were alive at the start of the present study. Of those, 258 (response 89%) were willing to participate and were visited in their home in order to collect data. Eighty-two (32%) participants were able to visit the study center and underwent a radiographic examination (Fig. 1). The majority of the study participants were women (74%) and, overall, participants were slightly overweight (BMI 27.0 kg/m^2). The frequency of "history of OA", derived from the general practitioner as well as innate cytokine production levels were equally distributed among those who were able and those who were unable to come to the study center (range of *P*-values: 0.23–0.86).

Absence of OA at 90 years

Thirteen (15.9%) participants met the criteria "free from OA". Hand OA was absent in 24 (29.3%) participants. The majority of participants had no knee ($n = 42$, 51.2%) nor hip ($n = 52$, 63.4%) OA.

Innate ex-vivo production of cytokines in 90-year-olds without OA

The mean difference between participants "free from OA" compared to those with OA in innate *ex-vivo* production of IL-1β was 3.1 (95% CI 0.7–6.6, $P = 0.007$) pg/ml lower in the former group; IL-6 levels were 11.7 (95% CI 0.9–24.5, $P = 0.03$) pg/ml lower; and IL-1RA levels were 8.1 (95% CI 1.5–16.2, $P = 0.014$) pg/ml lower. The mean innate *ex-vivo* production of IL-10 and TNF-α did not differ between the two groups.

Table I shows the association of the absence of OA in relation to tertiles of innate *ex-vivo* cytokine production. The OR's for "free from OA" was 11.2 [1.1–115.9] for participants in the lowest tertile of IL-1β production, and 6.7 [1.1–41.2] for those in the lowest tertile of IL-6 production (both in comparison to those in the highest tertile). All calculations were adjusted for gender and BMI. Furthermore, lower IL-1RA production was seen in absence of OA (OR 6.5 [0.95–44.5]). No associations were found for IL-10 and TNF-α.

The difference in cumulative distribution of innate cytokine production levels of IL-1β, IL-6 and IL-1RA is depicted in Fig. 2.

Due to the heterogenic nature of OA, we investigated whether these associations were joint site specific. Therefore, we compared absence of individual joint site OA with tertiles of IL-1β, IL-6 and IL-1RA production. The lowest tertiles of IL-6 and IL-1RA production were both associated with the absence of hand OA (OR 8.7 [1.9–39.5] and OR 4.4 [1.1–18.0] respectively). The lowest tertile of IL-1β production was associated with the absence of hip OA (OR 4.6 [1.1–18.5]).

Table I
Absence of OA in relation to tertiles of innate cytokine production

Cytokine, tertiles (pg/ml)	Absence of			Free from OA
	Hand OA	Knee OA	Hip OA	
IL-1β				
1.0–3.2	2.5 (0.7–9.2)	3.0 (0.8–10.4)	**4.6 (1.1–18.5)**	**11.3 (1.1–115.9)**
3.3–6.4	2.8 (0.8–10.7)	2.3 (0.6–8.2)	2.3 (0.6–8.5)	**13.2 (1.3–136.6)**
6.5–31.5	1.0	1.0	1.0	1.0
IL-6				
29.8–51.8	**8.7 (1.9–39.5)**	1.6 (0.5–5.8)	2.0 (0.5–8.1)	**6.7 (1.1–41.2)**
51.9–73.8	4.2 (0.9–20.4)	1.4 (0.4–5.1)	1.2 (0.3–4.4)	3.3 (0.4–23.9)
73.9–112.1	1.0	1.0	1.0	1.0
TNF-α				
5.7–9.3	1.0 (0.3–3.5)	1.9 (0.5–7.3)	2.0 (0.5–8.1)	1.3 (0.3–5.8)
9.4–12.1	0.6 (0.2–2.1)	0.5 (0.1–1.9)	0.9 (0.2–3.3)	0.3 (0.1–2.0)
12.2–18.5	1.0	1.0	1.0	1.0
IL-1RA				
17.1–31.8	**4.4 (1.1–18.0)**	1.8 (0.5–6.6)	1.2 (0.3–4.4)	6.5 (0.95–44.5)
31.9–43.3	2.8 (0.7–10.9)	0.4 (0.1–1.5)	1.3 (0.3–4.8)	2.9 (0.5–18.7)
43.4–148.0	1.0	1.0	1.0	1.0
IL-10				
0.37–0.70	1.7 (0.5–6.3)	2.2 (0.6–8.0)	2.0 (0.5–7.7)	3.2 (0.6–16.6)
0.71–0.88	2.3 (0.6–8.3)	0.8 (0.2–2.6)	1.5 (0.4–5.4)	2.2 (0.4–12.2)
0.89–1.99	1.0	1.0	1.0	1.0

OR's (95% CI) represent the relative probability for participants to be "free from (subtype) OA" compared to participants in the highest tertile of cytokine production, adjusted for gender and BMI.

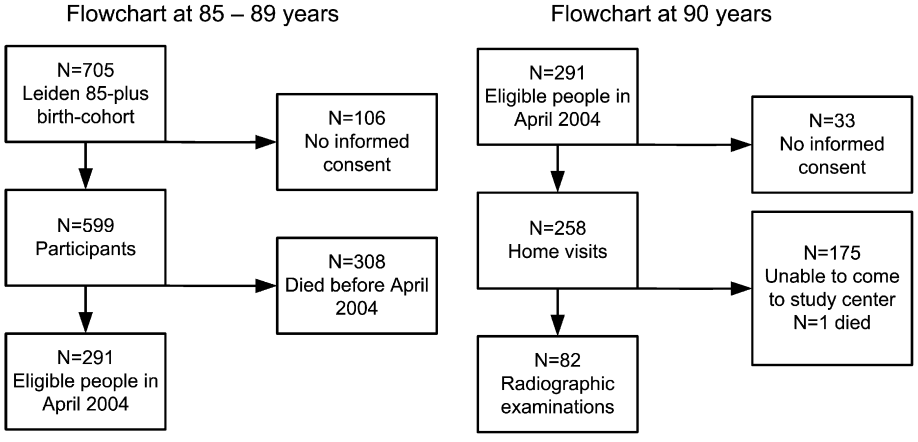

Fig. 1. Flowchart.

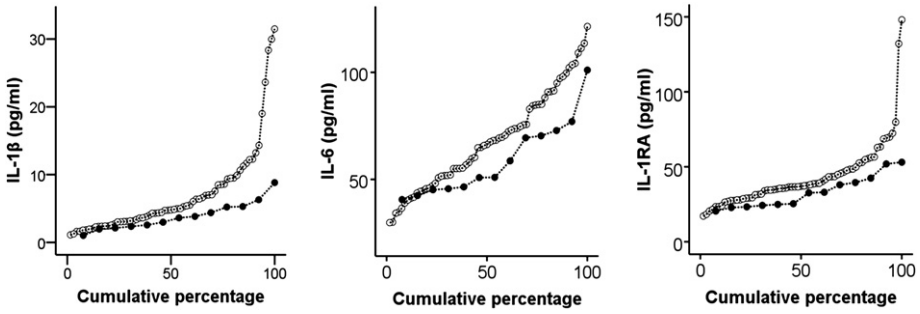

Fig. 2. Cumulative distribution of innate cytokine production levels of IL-1β, IL-6 and IL-1RA in participants "free from OA" (black dots) vs those with OA (open dots).

Discussion

The present study in 90-year-olds provides the first evidence that innate differences in the *ex-vivo* pro-inflammatory cytokine production are associated with absence of OA in old age. Participants with low innate production of IL-1β and IL-6 were found to have absence of OA more frequently, independent of gender and BMI. Joint site specific differences were observed; absence of hand OA was associated with low production of IL-6 and IL-1RA; absence of hip OA was associated with low production of IL-1β. No associations for IL-10 and TNF-α and absence of OA were demonstrated.

This unique cohort, in whom extensive radiographic, demographic, and immunologic data are available in 90-year-olds, gives way to investigate protective mechanisms against OA and the pathophysiology of OA in general. Population based studies including a range of age groups demonstrate that the prevalence and incidence of OA reaches a plateau or decrease above 70 years of age[18–21]. Possible explanations for the flattening of the prevalence above 70 years of age could be preferred early mortality in individuals with OA, or the existence of protective genetic factors against the development of OA that are associated with longevity. Some studies did indeed found an increased mortality risk for persons with OA[22–24]. In addition, genetic factors are thought to influence survival and longevity as well as the development of OA. So, genetic factors could possibly account for the observed declining prevalence of OA at old age[25]. Previously we have shown that a pro-inflammatory cytokine profile or the lack of an anti-inflammatory cytokine profile is associated with cardiovascular mortality or stroke respectively at old age[26,27]. Preferred mortality as well as protective genetic factors are therefore the most likely explanation for the observed flattening of the prevalence of OA.

Differences in cytokine characteristics have been shown to be associated with the incidence and progression of OA in epigenetic (innate cytokine production) as well as genetic (gene cluster polymorphisms) studies, suggesting a causal role in the pathogenesis of OA. IL-1β and TNF-α can stimulate their own production and induce chondrocytes and synovial cells to produce other cytokines, such as IL-8, IL-6, and leukocyte inhibitory factor (LIF), as well as stimulate proteases and prostaglandin E2 (PGE2) production. Moreover, IL-1β and TNF-α have also been shown to induce osteoclastic bone resorption *in vitro*, a phenomenon that may be involved in the remodeling of OA subchondral bone[5–7,9]. Animal studies have confirmed the essential role of IL-1β in cartilage destructive processes[28]. The role of IL-6 is less clear. It could contribute to OA's pathological process by (1) increasing the number of inflammatory cells in synovial tissue (2) stimulating the proliferation of chondrocytes; and (3) inducing an amplification of the IL-1 effects on the increased synthesis of matrix metalloproteases (MMP) and inhibiting proteoglycan production. In contrast, as IL-6 can induce production of tissue inhibitors of metalloproteinase (TIMP), and not MMP, it could also be involved in a feedback mechanism that limits proteolytic damage[18,29].

In the current study, we show that innate differences in the production of IL-1β and IL-6 are associated with the absence of OA in old age. Low innate production of IL-1β is associated with absence of OA, which is in line with the above mentioned studies. IL-1β is a catabolic cytokine causing damage; therefore, one could speculate that lower production of IL-1β to certain stimuli would lead to a state in which "joint-survival" following stress and stimuli is favored over "joint destruction".

Furthermore, low innate production of IL-6 is associated with the absence of OA, which is in inline with recent data on the association of IL-6 promotor variants and DIP OA[30]. Indeed we did see the strongest association for joint site specific OA and IL-6 in absence of hand OA. Low IL-1RA production tends to be associated with absence of OA, which is in line with our earlier observations at middle age[8]. The role of IL-10 in OA is unclear, and based on the current study, protection against OA is not the result of innate high IL-10 production.

Different pathophysiological processes account for heterogeneity in OA phenotypes. Therefore, different phenotypes were investigated for their individual association with innate cytokine production. We found that absence of hand OA was associated with innate low production of IL-6 and IL-1RA and that absence of hip OA was associated with low innate production of IL-1β. Associations were stronger for hand OA, which is in line with the genetic data from twin studies in OA that show that hand OA is more heritable than hip or knee OA[31,32].

This small hypothesis generating study has some potential limitations that should be addressed. First, participants who underwent radiographic examinations were a 'convenience sample' of our wider study population. However, the use of a 'convenience sample' did not influence the (general practitioner derived) prevalence of "history of OA" between those with vs those without radiographic examination. Nevertheless, we accept that participants with a radiographic examination and without OA may be overrepresented. This possible overrepresentation does however not harm the purpose of our explorative study; to determine protective factors against OA rather than risk factors in participants with OA. In fact an overrepresentation of absence of OA would reduce the effect size of the OR's. Second, the number of participants who underwent radiographic examination was small and therefore CIs are broad. Furthermore, it should be mentioned that the OR's overestimate true effect sizes in this small study with a common outcome parameter. However, despite the small sample size established risk factors, as well as new protective factors, for OA were statistically significant. Importantly, whole-blood samples were taken 5 years before determination of the phenotype. Since the cytokine levels reflect the capacity of innate cytokine production, which changes little over time[16], this will not influence the

results. Moreover, whole-blood samples were drawn at two different time point (age 85 and 86 years) enabling the use of the mean level of innate cytokine production, which strengthens our observations.

In conclusion, our study provides the first evidence that the low innate production of the pro-inflammatory cytokines IL-1β and IL-6 is associated with the absence of OA in old age. Absence of hand OA was associated with low IL-6 and low IL-1RA innate production and absence of hip OA was associated with low IL-1β production. These findings suggest the presence of protective factors against OA development and add to the body of evidence that cytokine pathways play a crucial, possibly causal, role in the pathophysiology of OA in general.

Conflict of interest

None.

Acknowledgements

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