The role of inflammation in muscle aging

Beenakker, K.G.M.

Chapter 5

Men have higher whole blood cytokine production responses than women: a pooled-analysis including 15 study populations

Submitted for publication by: K.G.M. Beenakker, R.G.J. Westendorp, A.J.M. de Craen, S. Chen, Y. Raz, B.E.P.B. Ballieux, R.G.H.H. Nelissen, A.F.L. Later, T.W. Huizinga, P.E. Slagboom, D.I. Boomsma, A.B. Maier.

Abstract

Incidence and prognosis of infectious diseases, sepsis, and auto-immune diseases differ between men and women. These differences have been attributed to sex differences in immune responsiveness. However, results from studies investigating sex differences in whole blood cytokine production response upon *ex-vivo* stimulation with lipopolysaccharide (LPS), a widely used assay for immune responsiveness, are inconclusive. The aim of the present study is to investigate sex differences in whole blood cytokine production response using cytokine data from 4020 subjects originating from 15 study populations, either from the general population or from patient populations with specific diseases. Men compared to women had a higher cytokine production response for tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-12, IL-1β, IL-1RA, IL-10, but not for interferon-γ. Granulocyte macrophage colony-stimulating factor production response was lower in men compared to women. These sex differences were independent of chronological age. As men had higher monocyte concentrations, we normalized cytokine production responses for monocyte concentration. After normalization sex differences in cytokine production response disappeared, except for IL-10, of which the production response was lower in men compared to women. In conclusion, men have higher whole blood cytokine production responses than women, which are largely explained by higher monocyte concentrations. A sex-based approach of handling immune responsiveness is crucial.

5.1 Introduction

Although incidence of bacterial infections (Klein, 2012; Washburn, Medearis & Childs, 1965; Offner, Moore & Biffl, 1999) and sepsis (Dombrovskiy *et al.*, 2007) are higher in men than in women, men less often develop auto-immune diseases (Beeson, 1994; Whitacre, 2001; Jacobson *et al.*, 1997) and have lower mortality risks in sepsis (Pietropaoli *et al.*, 2010; Nachtigall *et al.*, 2011; McLauchlan *et al.*, 1995; Eachempati, Hydo & Barie, 1999; Wichmann *et al.*, 2000; Schroder *et al.*, 1998). These clinical findings have been attributed to sex differences in immune responsiveness, which is hypothesized to be the evolutionary consequence of sex differences in reproductive strategies (Fish, 2008; McKean & Nunney, 2005).

Immune responsiveness can be assessed by a whole blood stimulation assay which measures the amount of cytokines produced by immune cells

upon stimulation with lipopolysaccharide (LPS) (Desch *et al.*, 1989; De Groote *et al.*, 1992). This stimulation assay is well reproducible (Van der Linden *et al.*, 1998), assesses primarily subjects cytokine production by monocytes (Damsgaard *et al.*, 2009a), which is under tight genetic control (De Craen *et al.*, 2005). A higher pro-inflammatory cytokine and a lower anti-inflammatory production response have been associated with survival from meningococcal infection (Preuss *et al.*, 2003), lower incidence of systemic lupus erythematosus (Van der Linden *et al.*, 2000), and less severe progression of multiple sclerosis (De Jong *et al.*, 2000, 2002). Several studies have reported a higher cytokine production response in men compared to women (Aulock *et al.*, 2006; Bruunsgaard *et al.*, 1999; Lefevre *et al.*, 2012; Kim-Fine *et al.*, 2012; Heesen *et al.*, 2002), although others have found no sex differences (Lynch, Dinarello & Cannon, 1994; Moxley *et al.*, 2004). Furthermore, these sex differences were only found in young and healthy subjects (Aulock *et al.*, 2006; Bruunsgaard *et al.*, 1999; Lefevre *et al.*, 2012; Kim-Fine *et al.*, 2012; Heesen *et al.*, 2002) and not in elderly (Bruunsgaard *et al.*, 1999; Moxley *et al.*, 2004). Moreover, no sex differences were found in studies in patients with specific diseases, such as patients after elective coronary stent placement (Rittersma *et al.*, 2005), severe blunt trauma (Majetschak *et al.*, 2000), or before and after abdominal surgery (Scheingraber *et al.*, 2005). However, all studies were performed with a small number of subjects and investigated only a limited number of cytokines which hampers a conclusive and comprehensive interpretation of sex differences in whole blood cytokine production response.

In the last 18 years the assay to measure whole blood cytokine production response has been performed in the same laboratory on a standardized manner using the same techniques for 15 study populations. This allowed us to perform a meta-analysis on sex differences in whole blood cytokine production response in an unprecedented large number of subjects. The included subjects were young, middle aged or elderly from the general population or had specific diseases like osteoarthritis, rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, or cardiac diseases warranting cardiac surgery. We first investigated the cytokine production response for tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-12, IL-1β, IL-1RA, IL-10, interferon-γ (IFN-γ) and granulocyte macrophage colony-stimulating factor (GM-CSF) in men compared to women. Second, we investigated whether sex differences in cytokine production response were different in various age groups. Third, we explored whether observed differences in cytokine produc-

tion response between men and women were attributable to differences in monocyte concentrations.

5.2 Methods

5.2.1 Study design

We included 15 study populations for which data on cytokine production response were generated in our laboratory over the last 18 years. Subject characteristics and key references for the study populations are given in 5.1.

5.2.2 Cytokine production response

Cytokine production response was measured using whole blood samples upon stimulation with LPS as described elsewhere Desch *et al.* (1989); Van der Linden *et al.* (1998). In short, venous blood samples were drawn and collected in heparinized tubes. Samples were then diluted with RPMI-1640 and incubated after addition of *E.Coli*-derived LPS at 37˚C and 5% CO_2 for 24 hours. Table 5.2 shows the used LPS concentration and period of incubation for each study population. After centrifugation, the supernatants were stored at −80˚C until assayed for TNF-α, IL-6, IL-1β, IL-10, and IFN-γ using Pelikine compact ELISA kits (Sanquin, Amsterdam, The Netherlands). IL-12, IL-1RA and GM-CSF were assayed using ELISA kits of R&D systems (Minneapolis, MN, USA). Before 2010 for IL-12 and IL-1RA ELISA kits of BioSource (Louvain, Belgium) were used. Table 5.2 shows the availability of the production response of each cytokine and the key references for additional available information on the material and methods for each study population. Subjects with an unstimulated TNF-α concentration above 100 μg/mL were excluded from further analysis due to concerns of possible contamination or acute infection (n = 27).

5.2.3 Hematologic measurements

Leukocyte, neutrophil, lymphocyte, monocyte, eosinophil and basophil concentrations were measured in venous blood samples using a Sysmex XE-2100 Hematology Analyzer (Sysmex, Etten-Leur, the Netherlands). Table 5.2 shows for which study populations these hematologic measurements were available.

5.2.4 Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics version 20 and StataCorp Stata/SE version 12.0. For each subject cytokine production response values were natural log (ln) transformed due to skewness. For each study population the difference in cytokine production response between men and women was calculated using linear regression analyses with log-transformed cytokine production response as dependent variable and sex and age as independent variables. Some study populations included subjects from the same family (Table 5.1). Linear regression analyses of data from these study populations were adjusted for family relationships between subjects by use of a family clustering factor as a sandwich estimator.

Random effect meta-analyses were performed to estimate the overall log-transformed difference in cytokine production response between men and women. The overall ratio of men over women in cytokine production response and its 95% confidence interval were calculated using an anti-log transformation of the overall log-transformed difference in cytokine production response between men and women and its 95% confidence interval (Bland & Altman, 1996).

Random effect meta-regression analyses were performed to investigate the effect of chronological age on the ratio of men over women in cytokine production response. These analyses were performed using study populations from the general population only so that the possible sex-specific effects of specific diseases and their treatments on age-related changes in cytokine production response could not affect our results. For these analyses each study population was stratified in age decades. The mean ratio of men over women in cytokine production response of pro-inflammatory cytokines was obtained by averaging the ratio of men over women in cytokine production response of TNF-α, IL-6, IL-12, and IL-1β. The mean ratio of men over women in cytokine production response of anti-inflammatory cytokines was obtained by averaging the ratio of men over women in cytokine production response of IL-1RA and IL-10. If data for a cytokine was missing, data on the available cytokines were used to calculate the mean ratio of men over women in cytokine production response.

To investigate the effect of normalization for monocyte concentration on sex differences in cytokine production response we normalized the levels of produced cytokines for the monocyte concentration. Normalization was performed by dividing the cytokine level in pg/L by the monocyte concentration in count $\times 10^9$/L.

5.3 Results

Table 5.1 shows the characteristics of the included study populations. Ten study populations included subjects from the general population (n = 3114) and five study populations included subjects with specific diseases (n = 906). The mean age of the subjects of the various study populations ranged from 21 to 85 years. The age of the individual subjects ranged from 3 to 95 years. The mean percentage of men of the study populations ranged from 18.6% to 74.2%.

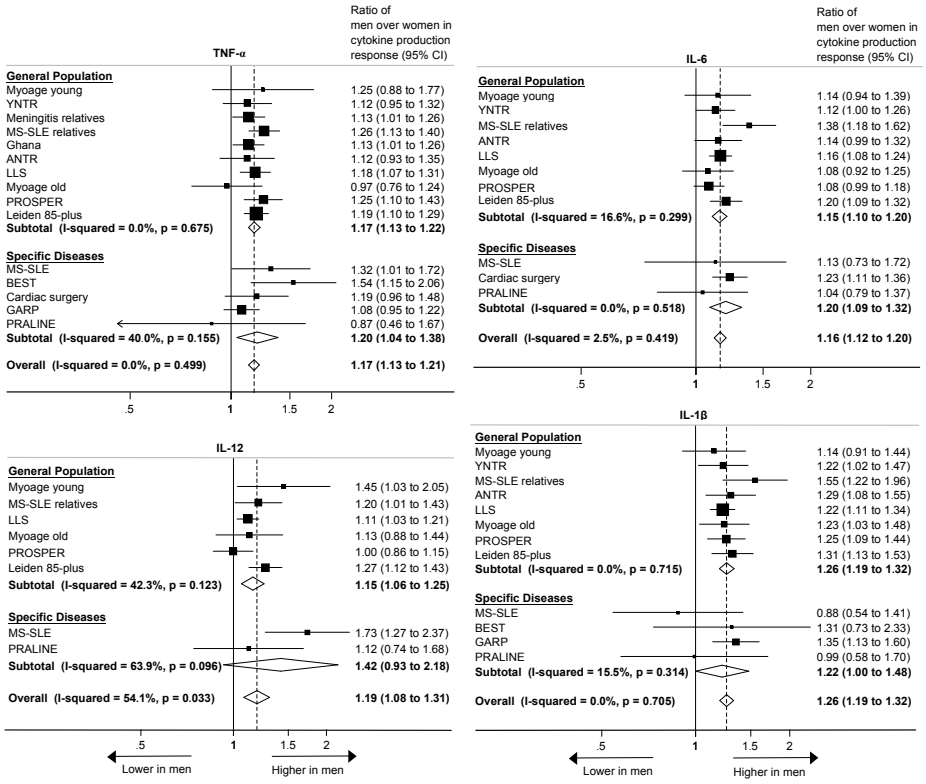

Figure 5.1. Forest plots of ratios of men over women in cytokine production response of pro-inflammatory cytokines.

Figure 5.1 shows forest plots of the ratios of men over women in cytokine production response for the pro-inflammatory cytokines TNF-α, IL-6, IL-12, and IL-1β. The cytokine production response of all cytokines was higher in men compared to women (all p-values < 0.001). Figure 5.2 shows the forest

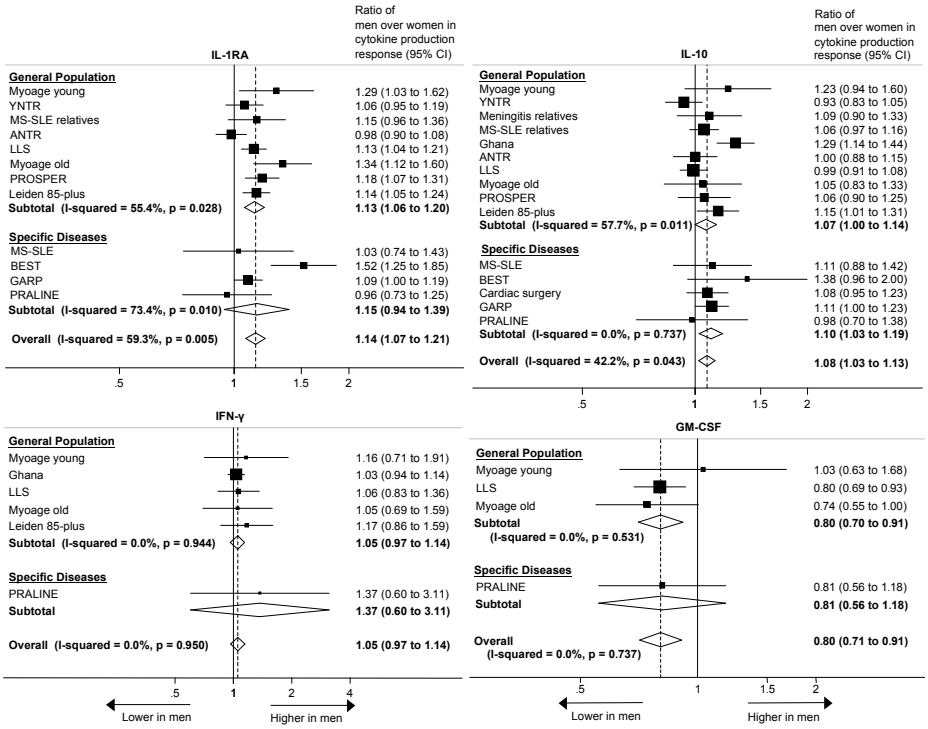

Figure 5.2. Forest plots of ratios of men over women in cytokine production response of anti-inflammatory and cytokines that stimulate pro-inflammatory cytokine production.

plots of the ratios of men over women in cytokine production response for the anti-inflammatory cytokines IL-1RA and IL-10 and the cytokines that stimulate pro-inflammatory cytokine production, IFN-γ and GM-CSF. The cytokine production response of IL-1RA and IL-10 was higher in men compared to women (p-values < 0.001 and < 0.05 respectively). No significant sex differences in cytokine production response of IFN-γ were found (p-value $= 0.23$). The cytokine production response of GM-CSF was lower in men compared to women (p-values < 0.001). Results from study populations including subjects from the general population were not significantly different from results from that including subjects with specific diseases. Noteworthy, an identical pattern was observed in the Ghana study including subjects from African decent living under adverse environmental conditions in Ghana and in studies including subjects from Western populations.

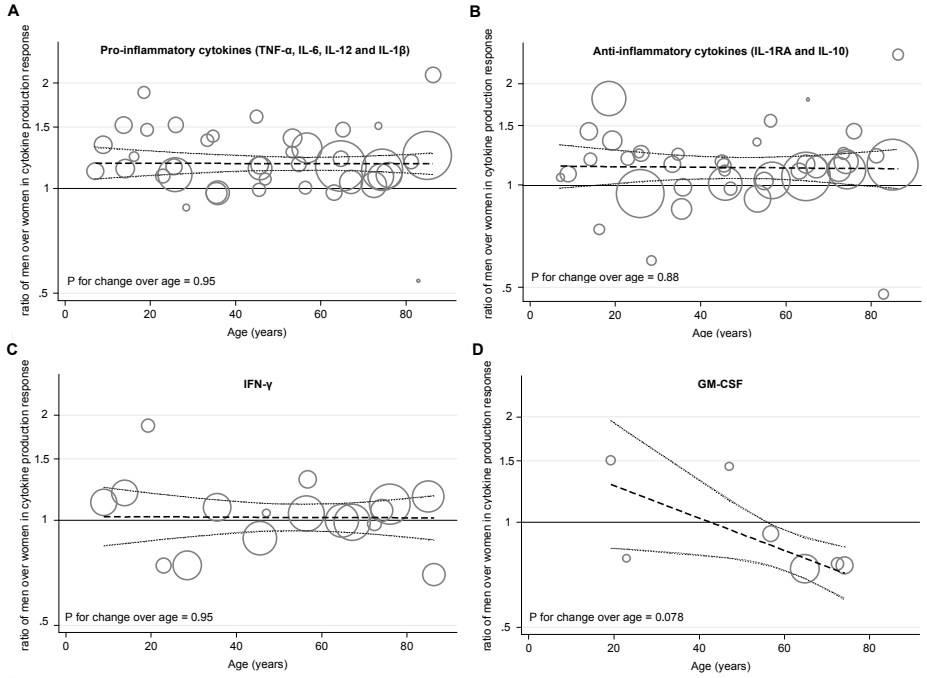

Figure 5.3. Ratios of men over women in cytokine production response dependent on chronological age.
Scatter plot of study populations from the general population stratified for age decades with meta-regression lines with 95% confidence interval. A larger circle size indicates a higher precision of the estimate (circle size = $1/SE^2$). An estimate represents the mean of the ratio of men over women in cytokine production response for (**A**) TNF-α, IL-6, IL-12 and IL-1β (n = 40), (**B**) IL-1RA and IL-10 (n = 40), (**C**) IFN-γ (n = 17), and (**D**) GM-CSF (n = 7).

Figure 5.3 shows the mean ratios of men over women in cytokine production response over chronological age for the study populations including subjects from the general population. The mean ratios of men over women in cytokine production response for the pro-inflammatory (IL-6, TNF-α, IL-1β, and IL-12) and anti-inflammatory cytokines (IL-10 and IL-1RA) as well as for IFN-γ did not change over age (p-values for change over age all > 0.87). The mean ratio of men over women in cytokine production response for GM-CSF was lower at higher ages, but not statistically significant (p value for change over age = 0.078).

In three of included studies, Myoage young, Myoage old and LLS, it was

possible to explore whether sex differences in cytokine production response were attributable to sex differences in monocyte concentrations due to the availability of data on leukocyte differential concentrations. Table 5.3 shows the pooled leukocyte differential concentrations in men and women. Leukocyte concentrations did not differ between men and women. Neutrophil and monocyte concentrations were higher in men compared to women ($p = 0.008$ and < 0.001 respectively), while lymphocyte concentration was lower in men compared to women ($p < 0.001$). Therefore, cytokine production response of the cytokines that are mainly produced by monocytes (IL-6, TNF-α, IL-1β, IL-12, IL-10, and IL-1RA) were normalized for the monocyte concentration for each subject. Table 5.4 shows that the pooled ratios of men over women in cytokine production response normalized for monocyte concentration for IL-6, TNF-α, IL-12, IL-1β, and IL-1RA were not significantly different from unity, indicating the absence of sex differences. The pooled ratio of men over women in cytokine production response normalized for monocyte concentration for IL-10 was 0.89 (95% CI 0.83 to 0.96).

5.4 Discussion

In the present study we showed that men compared to women had a higher cytokine production response upon stimulation with LPS for IL-6, TNF-α, IL-12, IL-1β, IL-10, and IL-1RA, but not IFN-γ. GM-CSF production response was lower in men. These sex differences did not differ over age. As men had higher monocyte concentrations, we normalized cytokine production responses for monocyte concentration. No sex differences in cytokine production response per monocyte were found, except of IL-10 which was significantly lower in men.

Our findings are in agreement with other studies reporting a higher cytokine production response in men compared to women (Aulock *et al.*, 2006; Bruunsgaard *et al.*, 1999; Lefevre *et al.*, 2012; Kim-Fine *et al.*, 2012; Heesen *et al.*, 2002; Auger *et al.*, 2011; Balteskard, Brox & Osterud, 1993; Beenakker *et al.*, 2013; Kimura *et al.*, 2008; Trilok-Kumar *et al.*, 2012). However, these studies were small and found differences only in relatively young and healthy subjects. Moreover, others did not find sex differences (Bruunsgaard *et al.*, 1999; Lefevre *et al.*, 2012; Moxley *et al.*, 2004; Rittersma *et al.*, 2005; Majetschak *et al.*, 2000; Scheingraber *et al.*, 2005; Azevedo *et al.*, 2005). We showed that sex differences in cytokine production response are present in healthy populations of all ages and are independent of specific diseases

like osteoarthritis, rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, or cardiac diseases warranting cardiac surgery. Moreover, we found the same pattern of sex differences in Ghanaians from African decent living under adverse environmental conditions in Ghana. These findings confirm sex differences in cytokine production response as a true biological phenomenon.

The measurement of whole blood cytokine production response is a model primarily designed to investigate immune responsiveness in sepsis. In patients with multiple injuries, a higher TNF-? and IL-6 production response on the first day of admission has been associated with a higher risk of developing severe sepsis (Majetschak *et al.*, 2000). We showed that TNF-α and IL-6 production response are higher in men compared to women, which could be one of the factors causing the higher incidence of sepsis in men compared to women (Dombrovskiy *et al.*, 2007), next to other factors like sex differences in tissue derived cytokine production and antigen exposure (Klein, 2000; Van Eijk *et al.*, 2007). In contrast, mortality risks in sepsis patients have shown to be lower in men compared to women (Pietropaoli *et al.*, 2010; Nachtigall *et al.*, 2011; McLauchlan *et al.*, 1995; Eachempati, Hydo & Barie, 1999; Wichmann *et al.*, 2000; Schroder *et al.*, 1998). This could be explained by the observation that the majority of the deaths in sepsis do not occur during the initial hyperinflammatory phase, but during the later immunosuppressive phase characterized by a reduced pro-inflammatory cytokine production response (Hotchkiss *et al.*, 2013). IL-1β production response during the late phase of sepsis has been shown to be higher in sepsis survivors compered to non-survivors (Weighardt *et al.*, 2000). We found a higher IL-1β production response in men compared women. However, we did not investigate whether this sex difference is still present during the late immunosuppressive phase in sepsis patients. If so, then this could be one of the factors causing the lower sepsis mortality risk in men compared to women.

The measurement of cytokine production response upon stimulation with LPS has also been used as model of immune responsiveness in non-infectious disease. A low cytokine production response has, on the one hand, been associated with consequences of the aging process like mortality (Wijsman *et al.*, 2011), metabolic syndrome, type 2 diabetes (Van Exel *et al.*, 2002), lower serum triiodothyronine levels (Rozing *et al.*, 2011), and low muscle mass and strength (Beenakker *et al.*, 2013). On the other hand, a low cytokine production response has been associated with familial traits like a negative family history of Alzheimer's disease (Van Exel *et al.*, 2009), a less severe

progression of osteoarthritis within sibling pairs with osteoarthritis at multiple sites (Botha-Scheepers *et al.*, 2008; Bijsterbosch *et al.*, 2014), and a negative family history of systemic lupus erythematosus (Van der Linden *et al.*, 2000). It is tempting to speculate about the contribution of sex differences in immune responsiveness in the sex differences in incidences and prognoses of these diseases. However, it still needs to be elucidated whether cytokine production response plays a causal role in the development or progression of these diseases.

This study is the first to describe the relation between chronological age and sex differences in cytokine production response. Sex differences are commonly attributed to the sex hormones and are expected to be reduced after menopause. However, we found that sex differences in cytokine production response are independent of chronological age and remained after menopause. These results are in contrast with one relatively small study showing that sex differences in TNF-α cytokine production response are present in young subjects and not in elderly (Bruunsgaard *et al.*, 1999). Moreover, experimental studies on the effects of oestrogen, progesterone, and testosterone on cytokine production response report conflicting results (Bouman, Heineman & Faas, 2005). Likely, sex differences are not caused by the activational (reversible) effects of sex hormones on monocytes, but by the organizational (permanent) effects of sex hormones on monocyte concentrations in the blood (Arnold, 2009). Alternatively, sex differences could also be independent of sex hormones and be caused by the effect of a sex-biased expression of X and Y genes in bone marrow cells (Arnold, 2009).

We found that the observed sex differences in cytokine production response are largely dependent on sex differences in monocyte concentration. In agreement with our findings, some have reported higher monocyte concentrations in men compared to women (Aulock *et al.*, 2006; Starr & Deary, 2011; McIlhagger *et al.*, 2010; Bouman *et al.*, 2004), although others did not found sex differences (Bain & England, 1975; Bain, 1996). Like Aulock *et al.* (2006), normalization of cytokine production responses for individual monocyte concentration largely cancelled sex differences for the majority of the cytokines in our study. It is currently unknown whether normalization of cytokine production response for individual monocyte concentration increases the clinical relevance of the measurement. One could argue that it is not relevant whether the cytokines are produced by a larger number of monocytes or by a larger production of cytokines per monocyte (Van der Linden *et al.*, 1998). This argument is especially applicable for studies investigating

the effect of cytokine production response on other circulating immune cells like lymphocytes, since produced cytokines influence these cells directly via their cytokine receptors. The same is true for the effect of cytokine production response on vascular endothelial cells, which are also known to have cytokine receptors that are able to induce vascular dysfunction (Sprague & Khalil, 2009). However, the argument might be less applicable for studies investigating diseases like cancer where the monocytes infiltrate the tissue. In these cases, the cytokine production response is dependent on the number of monocytes available to infiltrate, and therefore normalising the cytokine production response for individual monocyte concentration would be preferred.

Interestingly, we found that the IL-10 production response per monocyte is higher in women compared to men. In women, reproductive success has been described to be dependent on a high IL-10 production response at the foetal-maternal interface (Piccinni *et al.*, 1998; Westendorp *et al.*, 2001). Monocytes infiltrate into the uterine tissues during early gestation and significantly increase in number with the onset of labor (Gomez-Lopez, Guilbert & Olson, 2010). However, it is not known whether reproductive success associates with the number of infiltrating monocytes independent of their IL-10 production response.

The present study has several strengths. First, data from all individual subjects were available. Second, a large variety of cytokines were measured in a large variety of study populations, including subjects within a broad age range and with different health conditions. Third, all cytokines were measured in our laboratory or in cooperation with our laboratory in a standardized manner. A limitation is that cytokine production response was measured using a specific ligand secreted by bacteria. However, also other pathogens as well as trauma elicit the same inflammatory response and TLR-4, the pathogen recognition receptors (PRR) for LPS, is also known to recognize endogenous danger signals (Bianchi & Manfredi, 2009).

In conclusion, we showed that sex differences in immune responsiveness and leukocyte differential concentrations are profound and consistent. This underscores the notion that the immune system of men and women are distinctly different and underscores the importance of taking sex into account when studying immunologic processes. We postulate that a sex-based approach intervening the immune system is crucial for immune modulatory therapies.

5.5 Key references for Tables 5.1 and 5.2

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Table 5.1. Characteristics of the included study populations.

Study name	Key ref. for study design	Subjects (n)	Family clusters (n)	Characteristics subjects	Age mean (SD)	Men (%)
General population						
Myoage young	McP13	35	n/a	Young subjects	21·4 (2·7)	42·9
YNTR	Boo06	161	74	Young twins and their first degree relatives	27·0 (4·1)	47·2
Meningitis relatives	Wes97	180	60	First degree relatives of meningitis patients	30·9 (16·0)	48·3
MS-SLE relatives	Lin00	550	177	First degree relatives of MS and SLE patients	44·8 (16·9)	44·5
Ghana	May09	627	n/a	Population living under adverse environmental conditions in Ghana	47·2 (20·6)	26·5
ANRT	Boo06	140	64	Adult twins and first degree relatives	49·6 (6·9)	45·0
LLS	Bee13	392	322	Offspring of long-lived sibling and the spouses of the offspring	63·0 (6·5)	49·7
Myoage old	McP13	74	n/a	Relatively healthy and cognitively active elderly	74·2 (3·1)	51·4
PROSPER	She99	403	n/a	Elderly at risk for cardiovascular diseases	75·1 (3·3)	53·6
Leiden 85-plus	Wie02	552	n/a	Inhabitants of Leiden aged 85 years	85 (-)	33·2
Specific diseases						
MS-SLE	Lin00	152	n/a	Patients with MS or SLE	46·2 (12·8)	28·3
BEST	Goe05	73	n/a	RA patients before start with DMARDs	55·1 (15·1)	32·9
Cardiac surgery	Lat09	199	n/a	Patients before cardiac surgery	64·3 (11·6)	72·4
GARP	Riy05	370	188	OA patients and OA-relatives with joint complains	70·7 (7·6)	18·6
PRALINE	Sch16	112	n/a	Patients before knee replacement surgery	73·1 (9·9)	18·8

MS: multiple sclerosis, SLE: systemic lupus erythematosus, OA: osteoarthritis, RA: rheumatoid arthritis, Number of family structures: number of independent observations corrected for family relationships between subjects, n/a: not available. **See section 5.5 for the key references.**

Table 5.2. Method of whole blood stimulation and availability of cytokine production capacity per study population.

Name study population	Key ref. for methods	LPS conc.	Monocyte conc. available	Cytokine production response							
				TNF-α	IL-6	IL-12	IL-1β	IL-1RA	IL-10	IFN-γ	GM-CSF
General population											
Myoage young	n/a	10 µg/ml	v	v	v	v	v	v	v	v	v
YNTR	Cra05	10 ng/ml		v	v		v	v	v		
Meningitis relatives	Wes97	1 µg/ml		v[†]					v		
MS-SLE relatives	Lin00	10 ng/ml		v	v	v	v	v	v		
Ghana	May09	10 µg/ml		v					v	v	
ANTR	Cra05	10 ng/ml		v	v		v	v	v		
LLS	Bee13	10 ng/ml	v	v	v	v[§]	v	v	v	v	v[§]
Myoage old	n/a	10 µg/ml	v	v	v	v	v	v	v	v	v
PROSPER	Tro09	10 ng/ml		v	v	v	v	v	v		
Leiden 85-plus	Big04	10 ng/ml		v*	v	v	v	v	v	v	
Specific diseases											
MS-SLE	Lin00	10 ng/ml		v*	v	v[‡]	v	v	v		
BEST	Vri07	10 ng/ml		v			v	v	v		
Cardiac surgery	n/a	10 ng/ml		v	v				v		
GARP	Bot08	10 ng/ml		v*			v	v	v		
PRALINE	n/a	10 µg/ml		v	v	v	v	v	v	v	v

Ref.: Reference, Conc.: concentration, LPS concentration: whole blood samples were incubated for with lipopolysaccharide for 24 hours at 37°C. n/a: not available. * LPS was incubated for 4 hours, [†] LPS was incubated for 6 hours, [‡] 1µg/ml LPS was used, [§] 50 µg/ml LPS was used. **See section 5.5 for the key references.**

Table 5.3. Pooled leukocyte concentration and differentiation in men and women of three independent study populations (Myoage young, Myoage old and LLS).

	Men	Women	
	mean (95% CI)	mean (95% CI)	p-value
Leukocytes $*10^9$/L	6.08 (5.34 to 6.82)	5.87 (4.89 to 6.85)	0.29
Neutrophils $*10^9$/L	3.58 (3.08 to 4.08)	3.25 (2.56 to 3.94)	0.008
Lymphocytes $*10^9$/L	1.79 (1.58 to 2.00)	1.99 (1.73 to 2.25)	<0.001
Monocytes $*10^9$/L	0.53 (0.49 to 0.57)	0.46 (0.41 to 0.50)	<0.001
Eosinophils $*10^9$/L	0.15 (0.15 to 0.17)	0.15 (0.13 to 0.17)	0.12
Basophils $*10^9$/L	0.03 (0.02 to 0.03)	0.03 (0.02 to 0.04)	0.25

Estimates were calculated using a meta-analysis model with random effects and corrected for age and family relationships between subjects, CI: confidence interval.

Table 5.4. Pooled effect of monocyte concentration normalization on ratios of men over women in cytokine production response in three independent study populations (Myoage young, Myoage old and LLS).

	Not normalized for monocyte concentration		Normalized for monocyte concentration	
	Ratio of men over women in cytokine production response (95% CI)	p-value	Ratio of men over women in cytokine production response (95% CI)	p-value
TNF-α	1.14 (1.02 to 1.28)	0.021	0.98 (0.86 to 1.13)	0.79
IL-6	1.15 (1.08 to 1.22)	<0.001	0.98 (0.90 to 1.06)	0.61
IL-12	1.14 (1.04 to 1.24)	0.007	1.09 (1.00 to 1.20)	0.053
IL-1β	1.21 (1.12 to 1.31)	<0.001	1.06 (0.98 to 1.14)	0.17
IL-1RA	1.22 (1.08 to 1.38)	0.002	1.05 (0.92 to 1.19)	0.47
IL-10	1.03 (0.92 to 1.13)	0.59	0.89 (0.83 to 0.96)	0.001

Estimates were calculated using a meta-analysis model with random effects and corrected for age and family relationships between subjects, CI: confidence interval.