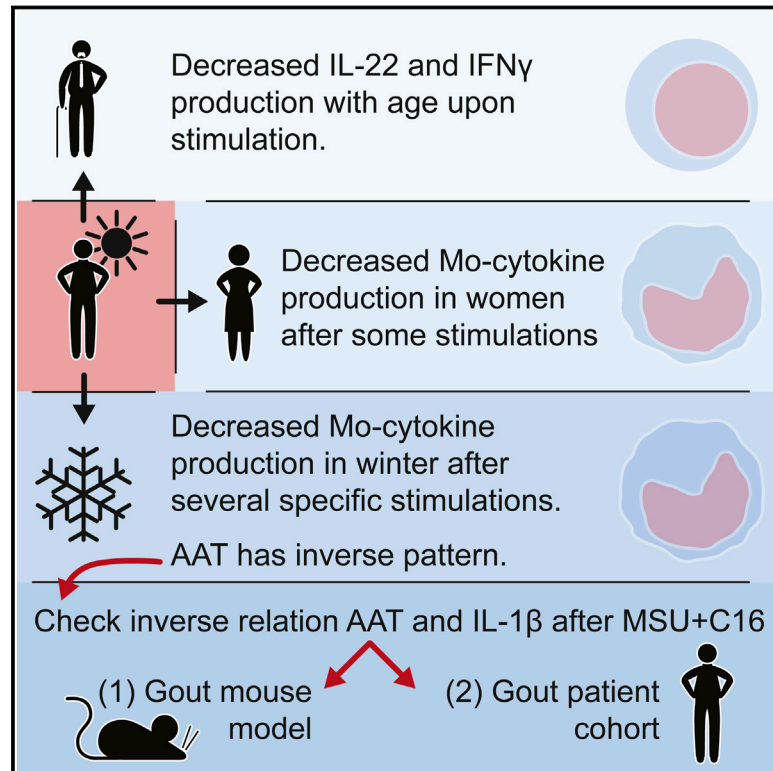# Cell

Host and Environmental Factors Influencing Individual Human Cytokine Responses

Graphical Abstract

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In Brief

As part of the Human Functional Genomics Project, mapping of environmental and non-genetic host factors reveals critical associations between age, gender, and annual seasonality in inter-individual variability of immune cell function.

Highlights

- HFGP cohort: host/environment, genetics, and microbiome affect cytokine production

- IFN-γ and IL-22, but not IL-17 and Mo-derived cytokine responses, decrease with age

- Gender affects cytokine responses, resulting in disease susceptibility differences

- Cytokine responses are season dependent, influenced by AAT concentrations

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Host and Environmental Factors Influencing Individual Human Cytokine Responses

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SUMMARY

Differences in susceptibility to immune-mediated diseases are determined by variability in immune responses. In three studies within the Human Functional Genomics Project, we assessed the effect of environmental and non-genetic host factors of the genetic make-up of the host and of the intestinal microbiome on the cytokine responses in humans. We analyzed the association of these factors with circulating mediators and with six cytokines after stimulation with 19 bacterial, fungal, viral, and non-microbial metabolic stimuli in 534 healthy subjects. In this first study, we show a strong impact of non-genetic host factors (e.g., age and gender) on cytokine production and circulating mediators. Additionally, annual seasonality is found to be an important environmental factor influencing cytokine production. Alpha-1-antitrypsin concentrations partially mediate the seasonality of cytokine responses, whereas the effect of vitamin D levels is limited. The complete dataset has been made publicly available as a comprehensive resource for future studies.

INTRODUCTION

Host defense mechanisms mediated by the immune system protect the host from the invading pathogens that continuously attempt to breach the mucosal and skin barriers. Individuals with diminished immune responses from inborn or acquired causes have an increased susceptibility to infections (Blot et al., 2002; Clark and Hajjeh, 2002; Fishman, 2007; Fishman and Rubin, 1998; McNeil et al., 2001). Conversely, individuals with an overactive immune system are more susceptible to autoimmune diseases such as rheumatoid arthritis, type 1 diabetes and multiple sclerosis, and inflammatory diseases such as gout, Crohn's disease, and atherosclerosis (Gandhi et al., 2010; Martinon, 2010; Szablewski, 2014). Variability in the immune responses also influences susceptibility to other important pathologies such as malignant processes (de Visser et al., 2006) and neurodegenerative diseases like Parkinson's disease and Alzheimer's disease (Heneka et al., 2015; Mosley et al., 2012). This variability in immune responses is likely affected by factors already known to influence disease prevalence such as age, gender, and seasonality. For instance, women are more likely to suffer from autoimmune diseases, it gets more difficult to fight off infections with age, and influenza infections peak in winter. However, the differences in susceptibility to immune-mediated diseases between individuals cannot be fully accounted for by what is currently known, and a systems biology-based approach
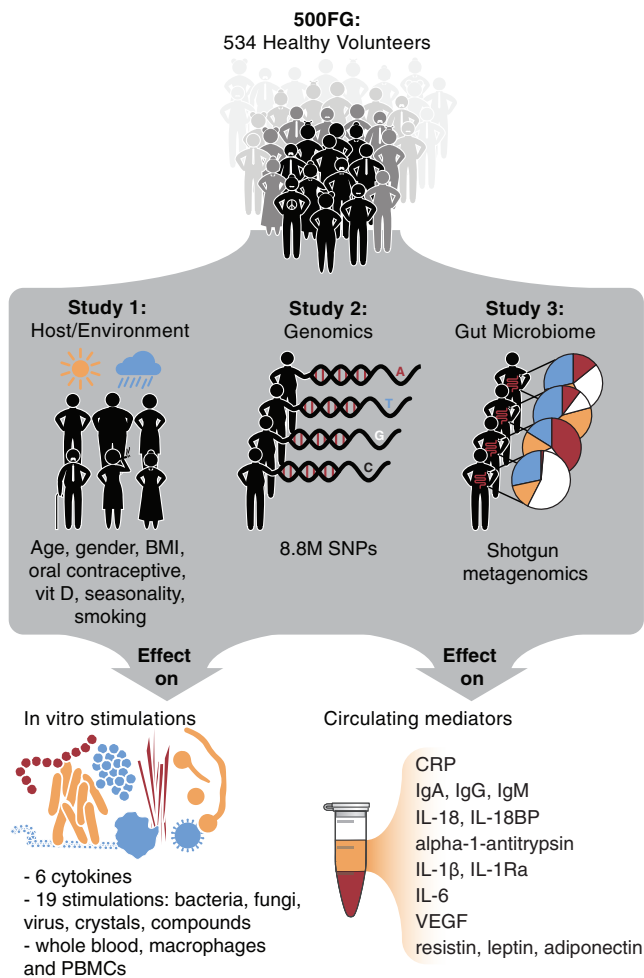
Figure 1. Schematic Overview of the Three Studies on the 500FG Cohort

The cohort consists of 534 volunteers with varying characteristics. The current manuscript is the first in a series of three studies presented in this issue of *Cell*, that aim to provide a systematic assessment of the impact of various intrinsic and environmental factors (this manuscript), the host genome (Li et al., 2016), and the gut microbiome (Schirmer et al., 2016) on cytokine production and baseline immune parameters in a systems biology-based approach. See also Figure S1 and Tables S1 and S2.

to comprehensively assess the environmental and host-related factors that influence immune responses is needed.

Production and release of proinflammatory cytokines is one of the most important components of host defense mechanisms, representing the communication network within the immune system. So far, variation of cytokine production capacity in the general population has been investigated only in small studies, and this limitation resulted in conflicting conclusions (Aulock et al., 2006; Bernstein and Murasko, 1998; Grandgirard et al., 2013; Hwang et al., 2015; Nielsen et al., 2013; Scott et al., 2013). The only large-scale studies of the immune system published to date are genome-wide studies focused on the regulatory effect of genetic variation on cytokine gene transcription levels (eQTLs) rather than on protein expression levels (Berry et al., 2010; Fair-

fax et al., 2014; Lee et al., 2014; Raj et al., 2014; Ye et al., 2014). These studies were based on a limited number of stimulations or used unstimulated cells. The few studies assessing variability of cytokine production are all based on responses to standard immune stimuli such as lipopolysaccharide (LPS) (Fairfax et al., 2014; Lee et al., 2014). A comprehensive assessment of environmental and host factors influencing cytokine responses to a wide range of pathological and physiological stimuli is still lacking.

The Human Functional Genomics Project (HFGP; www.humanfunctionalgenomics.org) aims to fill this gap. Within HFGP, the first major study that intends to assess the variability of human cytokine responses is the 500 Functional Genomics (500FG) study, comprising of a group of ∼500 healthy volunteers of Western-European ancestry. The specific aim of the 500FG cohort is to assess and integrate the various factors influencing individual human cytokine responses. The 500FG study has two important advantages compared to previous studies: first, it comprises the largest cohort of healthy individuals studied to date, and second, the cytokine production was assessed in response to a large panel of microbial and metabolic stimuli and in three different cellular systems. The current manuscript is the first in a series of three studies presented in this issue of *Cell*, that aim to provide a systematic assessment of the impact of various intrinsic and environmental factors (this manuscript), the host genome (Li et al., 2016), and the gut microbiome (Schirmer et al., 2016), on baseline immune status and cytokine production in a systems biology-based approach. A schematic overview of these three studies on the 500FG cohort is presented in Figure 1.

In this first study, we focus on environmental and non-genetic host factors that have been described to influence the immune response and/or disease prevalence (e.g., age, gender, BMI, oral contraceptive usage, smoking, vitamin D, and seasonality), but for which a comprehensive assessment of their effect on cytokine responses is missing. We observe that several of the studied factors directly influence immune parameters and host defense and we describe a novel vitamin D-independent/alpha-1-antitrypsin (AAT)-dependent effect of seasonality on inflammation.

RESULTS

Measurement of Circulating Immune Parameters

To study variation in baseline immune status, we measured circulating concentrations of some of the most important families of parameters responsible for host defense in the circulation: acute phase proteins (CRP and AAT), immunoglobulins (IgA, IgM, IgG, and 4 IgG+ sub-classes), adipokines (leptin, adiponectin, and resistin), IL-6, IL-18, IL-18-binding protein (IL-18BP), IL-1β, and interleukin-1 receptor antagonist (IL-1Ra). Measurement of resting levels of low abundance cytokines such as IL-1β, IL-6, IL-18, and VEGF are below the lower limit of quantification by standard ELISAs in a healthy cohort; therefore, the Ella microfluidic analyzer was used to assess cytokine concentrations in the fg/mL to low pg/mL range. For instance, the mean level of IL-6 concentrations of the cohort was 1.25 ± 0.06 pg/mL (range 0.15–8.1 pg/mL; see the STAR Methods for levels of all circulating mediators). The assessment of these parameters provides
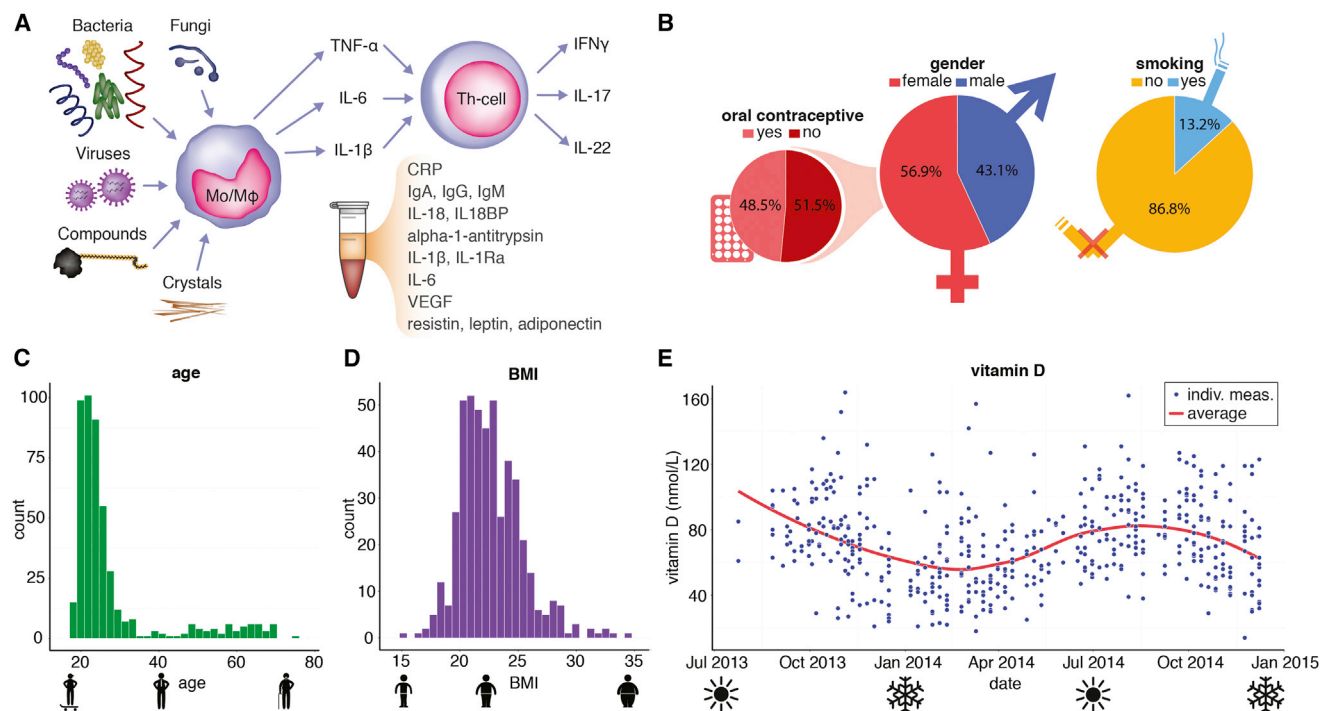
Figure 2. Schematic Depiction of the Study Parameters
(A) Stimuli and measurements in this study.
(B) Pie charts showing some characteristics of our cohort; from left to right, the percentage of women using oral contraceptives, the percentage of men and women, and the percentage of people that are active smokers.
(C) Histogram showing the age distribution in our cohort.
(D) Histogram showing the BMI distribution in our cohort.
(E) Scatterplot of the vitamin D levels for all individuals plotted against the date which they provided blood samples. The blue dots are the individual vitamin D measurements and the red line is the LOESS fit through these points.
See also Figure S1 and Tables S1 and S2.

a comprehensive view of baseline immune characteristics, most importantly of inflammatory status and humoral immunity.

The immune parameters were analyzed as a function of a set of environmental and intrinsic non-genetic host factors (age, gender, BMI, oral contraceptive usage, smoking, vitamin D concentrations, and seasonality) (Figure 2).

Correlations between Circulating Cytokine Concentrations Indicate Co-regulation

As displayed in Figures 3A, 3B, and S2A, some cytokine levels show positive correlations with other cytokine levels. IL-1β concentrations show a strong correlation with IL-6 ($p < 0.0001$), a particularly relevant observation, because treatment of patients with IL-1β blocking therapies reveals a consistent fall in circulating IL-6 levels (Dinarello et al., 2012). IL-1Ra and IL-6 concentrations also correlate significantly because these are both markers of inflammation (Figure 3B). IL-1β circulating concentrations also correlated with IL-1Ra ($p < 0.05$), and this is in agreement with in vitro and human trial data demonstrating that IL-1β induces its own receptor antagonist. In line with the well-known anti-inflammatory effects of AAT (Bergin et al., 2012; Lewis, 2012), the only strong negative correlation was found between AAT and IL-1β concentrations (Figure 3B).

Age and Gender Have a Significant Impact on Circulating Mediators

The concentrations of IL-6 and IL-1Ra are increased in the circulation of older individuals (Figure 3C, with relevant examples in Figure 3D). An increased low-level inflammation during the aging process (inflammaging) has been proposed as a culprit for metabolic syndrome and cardiovascular diseases (Kovacic et al., 2011a, 2011b; Veronica and Esther, 2012), and in our study, the higher IL-6 and IL-1Ra concentrations with age gives weight to this hypothesis. The effect of age on IL-6 levels is also supported by the validation of these findings in a second independent cohort of 300 individuals from the HFGP ($p = 1.12e-03$, Figure S2E). With regard to gender, we find significantly higher circulating concentrations of IL-1Ra in women. This was previously observed in a cohort of type II diabetes patients (Ybarra et al., 2008), which we now confirm in healthy individuals.

We also observe an increase in IL-18BP concentrations in older individuals (Figure 3C), and IL-18BP levels were also significantly higher in men than in women, while total IL-18 concentrations remained unaffected by both age and gender. However, in patients with cardiovascular disease, IL-18 levels are higher in men than in women (Opstad et al., 2011).
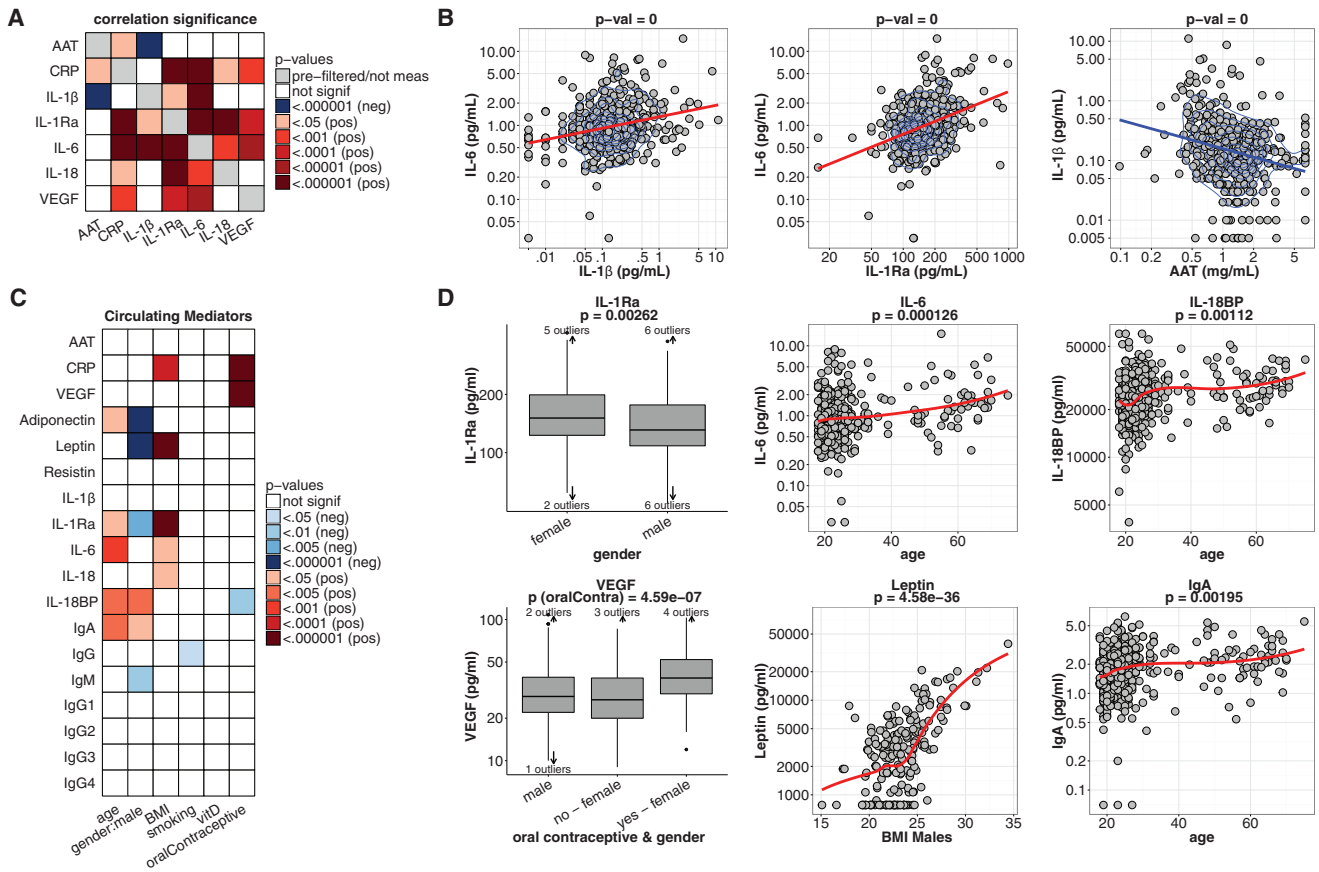
Figure 3. Correlations of Circulating Mediators with Non-genetic Host Characteristics

(A) The p values (FDR corrected) of the correlations of mediators with each other. For color codes of the FDR, see legend.

(B) Scatterplots of highly significant correlations from (A). The thin blue lines are contour lines, indicating the density of the scatterplot.

(C) Significance (FDR corrected) of the relation between host characteristics/environmental factors (x axis) to circulating mediators and immunoglobulins (y axis).

(D) Scatterplots showing examples of the effect of age, gender, oral contraceptive and BMI. The lines indicate the local regression (LOESS) fit.

See also Figure S2 and Table S3.

IgA immunoglobulin concentrations also increase with age (Figure 3C), as was previously detected in saliva (Gonzalez-Quintela et al., 2008; Jafarzadeh et al., 2010). IgA has been implicated with several age-related diseases such as macular degeneration and diabetes, which makes IgA a potential therapeutic agent for prophylaxis and/or treatment (Rodriguez-Segade et al., 1996; Yu et al., 2016). Higher IgA was also detected in men compared to women, as supported by earlier studies, while circulating concentrations of IgM were higher in women (Cassidy et al., 1974; Obiandu et al., 2013; Weber-Mzell et al., 2004). In another study from the HFGP (Aguirre-Gamboa et al., 2016), IgG2 and IgG4 are shown to significantly interact with age and gender, respectively. Due to the larger number of factors corrected for, and stronger multiple testing correction applied in this manuscript, we observe only a borderline significant effect. However, using similar corrections we find false discovery rates (FDRs) of 4.06e-3 and 2.20e-3 for IgG2 and IgG4, respectively.

Concentrations of leptin and adiponectin are higher in women than in men (Figure 3C). This is due to the higher percentage of adipose tissue in females compared to males, as well as a higher

secretion rate of leptin in females compared to males as previously shown by others (Böttner et al., 2004; Considine et al., 1996; Hellström et al., 2000). Adiponectin levels were also raised in older individuals. CRP levels show a positive association with the use of oral contraceptives, as shown by several earlier studies (Buchbinder et al., 2008; Cauci et al., 2008; van Rooijen et al., 2006). In addition, plasma VEGF was higher in women using oral contraceptives (Charnock-Jones et al., 2000; Macpherson et al., 1999) (Figure 3C), whereas circulating IL-18BP were lower. These findings provide clinical validation that biomarkers such as circulating low abundance cytokines reflect fundamental physiologic parameters in the absence of disease.

Stimulation of Proinflammatory Cytokines in Three Ex Vivo Systems

To comprehensively capture cytokine responses of immune cells, we measured the production of both monocyte-derived (IL-1β, TNF-α, IL-6) and lymphocyte-derived cytokines (IFNγ, IL-17, IL-22) after stimulation in three ex vivo systems (whole blood, peripheral blood mononuclear cells [PBMCs], and

monocyte-derived macrophages) with 1 of 19 stimuli (eight bacterial, four fungal, one virus, four purified microbial ligands, and two metabolic stimuli). We used these complex cellular systems with 24-hr (monocyte-derived cytokines) and 7-day (lymphocyte-derived cytokines) stimulation times in order to mimic real-life situations, as opposed to artificial systems using purified cell populations. This resulted in a total of 128 cytokine measurements for each of the 534 individuals included in this study. This is by far the largest set of stimuli-cytokine combinations measured to date, with previous studies generally studying one or two stimuli in one experimental setting. In addition, the choice was made to focus on protein levels (that confer biological activity) rather than gene expression (as in earlier eQTL studies), because protein levels are a better representation of an individual's immune state. Gene expression and protein expression levels of cytokines are not always correlated to one another, because post-transcriptional processes play a crucial role in determining the rate of cytokine synthesis and release (Anderson, 2008). A complete list of measurements is provided in Table S1, and example distributions are shown in Figure S1. The data for each participant (including all measurements of circulating immune parameters) can be found at https://hfgp.bbmri.nl/ (database is described in detail in the STAR Methods).

IFNγ and IL-22 Responses Decrease with Age

Production of monocyte-derived cytokines (IL-1β, TNF-α, and IL-6) after stimulation was similar across the ages of the volunteers, with the exception of *Staphylococcus aureus*-induced IL-1β and *Candida albicans* hyphae-induced IL-6, that showed a moderately higher production in the elderly. In contrast, there was a consistent effect of age on lymphocyte function, with the production of IFNγ and IL-22 being significantly lower in elderly individuals after stimulation with most pathogens, in line with the concept of immune-senescence (Baylis et al., 2013). Interestingly, no such impairment with age was observed for the production of IL-17, an important product of Th17 cells. The strongest decrease of cytokine production in the older participants was observed after stimulation with *Borrelia spp*., as detailed and analyzed in the HFGP manuscript by Oosting et al. (2016). The impact of age on cytokine production capacity induced by various microbial and metabolic stimuli is schematically depicted in Figure 4A, with relevant examples provided in Figure 4C.

Monocyte-Derived Cytokine Responses Are Increased in Men, whereas Women Have Increased Th17 Responses to *Candida*

The production of proinflammatory cytokines released from monocytes was higher in men after stimulation with several stimuli. In the whole-blood system, these cytokines were increased in men after LPS stimulation, while in PBMCs this effect was apparent especially after stimulation of *C. albicans* conidia. Figure 4B presents an overview of the gender effects on cytokine production, with examples shown in Figure 4E. Although the use of oral contraceptives did not have strong effects on cytokine production capacity in vitro, women using oral contraceptives did show an even further decreased IFNγ and TNF-α

response after LPS stimulation (Figures 4D and 4E). Th17 responses were mostly similar between men and women, although IL-17 and IL-22 production was higher in women after stimulation with *C. albicans* hyphae (Figure 4B).

Hormonal Differences Do Not Explain Gender-Specific Immune Differences

To investigate whether differences in circulating hormone levels may have a role in the above-described gender effects, we assessed the correlation of inflammatory markers with the levels of progesterone and testosterone in men and women separately. The majority of the cytokines and mediators identified to differ between men and women showed no correlation with progesterone and testosterone concentrations, excluding a potential role of hormones in explaining the gender differences. However, we identified several notable exceptions, with the significant correlations displayed in Figure 4F (all correlations in Figure S2C). One of the most important findings is that leptin concentrations show a clear negative correlation with testosterone levels within the male subgroup of the 500FG cohort (R = −0.36, FDR = 6.78e-06, see Figure 4G), supported by previous reports (Behre et al., 1997). Surprisingly, however, no such effect is observed in women. In fact, if anything, there is a positive correlation between leptin levels and testosterone (FDR = 0.07; Figure S2D), in line with a study that observed a similar effect for non-obese women (Soderberg et al., 2001). Moreover, there was a significant positive correlation and a significant negative correlation of testosterone and progesterone levels, respectively, with circulating IL-18BP concentrations in men, which was partially able to explain the gender difference.

Smoking and BMI Do Not Affect In Vitro Cytokine Production, but BMI Influences Several Circulating Mediators

We have analyzed two sets of parameters in relation with nongenetic host factors: resting circulating concentrations of the immune mediators and in-vitro cytokine production capacity after microbial stimulation. Surprisingly, with the exception of a few spurious effects, BMI and smoking had no detectable effect on in vitro cytokine production (Figures S3A and S3B), even though they are thought to be important modulators of immune responses (McCrea et al., 1994; Sopori, 2002). In contrast, some of the circulating mediators measured were significantly related to BMI (Figure 3C). As expected, leptin and CRP correlated positively with BMI (Buchbinder et al., 2008; Cauci et al., 2008; van Rooijen et al., 2006). BMI also showed a small but significant association with circulating levels of both IL-6 and IL-18, which is in accordance with the concept of an increased inflammatory status when BMI increases (Kantor et al., 2013; Khaodhiar et al., 2004; Siervo et al., 2012). This finding was confirmed in an independent cohort of volunteers (p = 4.3e-5, Figure S2A). The same increased inflammation is likely the cause of the increased IL-1Ra concentrations in individuals with high BMI: the levels of the cytokine are probably reactively upregulated. The only effect we observe for smoking is a reduction in IgG levels, which confirms the findings of a study by Gonzalez-Quintela et al. (2008).
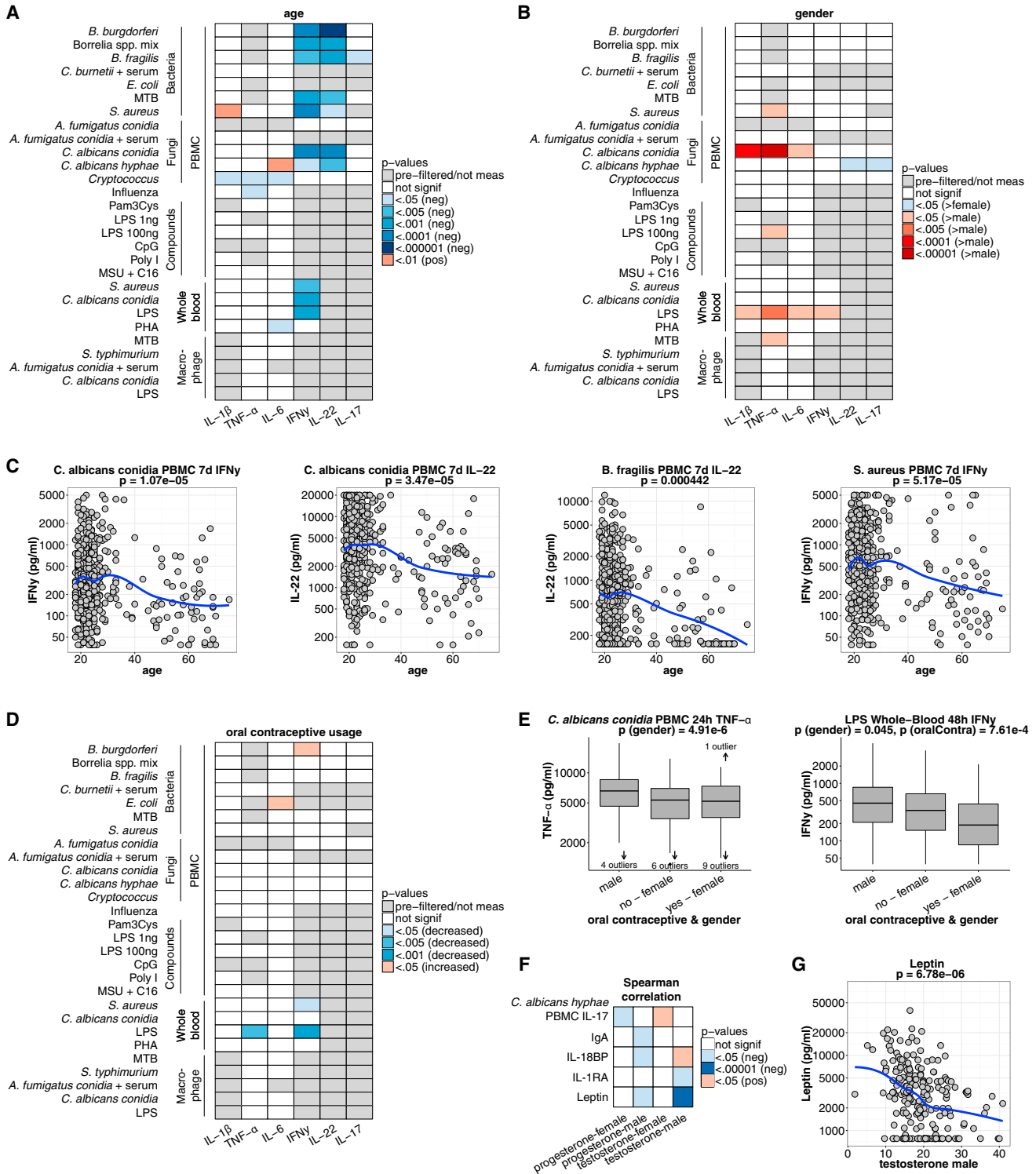
Figure 4. Relation of Age, Gender, and Oral Contraceptive Usage to Cytokine Production

(A) Significance of age in relation to different cytokines (x axis) induced by different stimuli (y axis), all values have been FDR-corrected. The darker the color, the greater the significance, where a decrease with age is blue and an increase is red (see figure legend).

(B) Similar plot to Figure 3A for gender. Red indicates a stronger response in men, whereas blue indicates a stronger response in women.

(C) Specific correlations of age to IFNγ and IL-22 production with different stimuli (the lines indicate the LOESS fit).

(D) Similar to Figure 4A and 3B, for the effect of "oral contraceptive usage," where red indicates an increase with usage of oral contraceptives and blue a decrease.

(legend continued on next page)

Seasonality Has a Major Impact on Cytokine Responses and Inflammation

It was recently suggested that gene expression in human immune cells shows annual seasonality (De Jong et al., 2014; Dopico et al., 2015). We therefore checked immune responses for the presence of annual seasonal patterns using linear regression. This analysis provided both the significance of the seasonality pattern and the month at which the immune responses were at their highest (Table S3). Results were confirmed using an independent nonlinear fitting method (Table S4). The results show that the production of several cytokines (TNF-α, IL-1β, and IL-6) shows a significant peak in summer (Figure 5A), while circulating AAT concentrations were highest in the winter (Figure 5C). The clearest effects of seasonality are apparent for monocyte-derived cytokines after stimulation with influenza virus, *Coxiella burnetti*, or *Cryptococcus neoformans* (Figure 5D). As influenza incidence displays a very clear seasonality pattern, with increased transmission in the winter months (Lipsitch and Viboud, 2009; Lofgren et al., 2007), it is tempting to speculate that the lower cytokine responses to influenza in the winter months may represent an important pathophysiological factor in this phenomenon.

The extent to which seasonal variation in baseline gene expression could contribute to seasonal variation in cytokine responses remains to be fully tested. To get an estimation of this contribution, we performed RNA-sequencing in a subset of 88 volunteers and first analyzed the seasonality patterns of AAT and the three cytokines showing seasonal responses (IL-1β, TNF-α, IL-6; Figure 4B). Only TNF-α mRNA showed a seasonal pattern of expression, peaking in summer, which matches protein secretion (Table S5). Additionally, we checked the same genes in a large pediatric cohort from Germany, which shares a very similar climate with the Netherlands (Dopico et al., 2015). PBMC expression of *SERPINA1* (the gene encoding AAT) was found to be seasonal in that cohort. It is important to note that AAT seasonality is among the weaker seasonal genes in the German cohort of Dopico et al., (2015) in which 5,136 genes were found to be seasonal (corresponding to ~1/4 of protein-coding genes in the human genome). Second, we assessed seasonality of mRNA expression of genes known to be involved in regulating cytokine production: PRRs, signaling molecules, and transcription factors (for details see the STAR Methods). Expression analysis of these genes in our cohort showed no individual seasonal patterns after multiple-testing correction (Table S6). Interestingly, in the genes significant before correction, there was an enrichment of seasonal genes peaking in summer (19 out of 30 genes, chi-square p < 0.01), the same season at which concentrations are highest for most seasonal cytokines. Inspection of this same regulatory set of genes in the larger German pediatric cohort revealed a larger set of seasonal genes involved in the regulation of cytokine expression (Table S7). Seasonality of these regulatory genes, combined with marginal seasonal gene expression of AAT and one out of three seasonal

cytokines, does point toward some transcriptional regulation. However, this also suggests that post-transcriptional processes, rather than gene transcription, are influenced strongly by seasonal variations. Additionally, the biological relevance of RNA levels is less direct, and more difficult to interpret, than protein expression levels. This strengthens our choice to investigate cytokine expression as a more direct measure of immune regulation.

Variations in Vitamin D Concentrations Have Limited Effect on Cytokine Production Capacity

Vitamin D has been repeatedly reported to have important immunomodulatory effects (Bikle, 2009; Correale et al., 2009), and based on this, we expected to observe important effects on cytokine production capacity. Surprisingly, vitamin D circulating concentrations did not have a significant effect on any of the cytokine production systems investigated here (Figure 5B). The seasonality of vitamin D has been suggested to influence inflammatory markers (Prietl et al., 2013): however, when we separated out the general periodicity over time and the residual variation of vitamin D at each time point, it was only the periodicity that was significantly correlated with cytokine outcomes and not the season-independent vitamin D variations (Figures S3C and S3D). This is not due to a lack of residual variation around the periodic signal, because the residuals have an amplitude similar to the periodic signal (Figure 2E). With inter- and intra-assay coefficients of variation of <5%, the residuals can also not be ascribed to measurement noise. There are a number of environmental factors that show seasonal variations, which explains why vitamin D concentrations alone are not a strong predictor of cytokine responses. Examples of factors that peaked in summer include temperature and atmospheric NH_3 and O_2 levels, whereas humidity and SO_2, NO, NO_2, and CO concentrations peaked in winter (Figure S4). Pollen, which is a known allergen, peaked in concentration at different times during our study depending on the species and could also have contributed to seasonal immune responses (examples in Figure S4). It is thus likely that vitamin D is only one of many other factors with a seasonal pattern that influence the immune response.

Alpha-1-Antitrypsin Is Partially Responsible for Seasonality of Cytokine Responses: Impact on Gouty Inflammation

In contrast to in vitro cytokine production, most circulating markers were not influenced by the season, with the notable exception of plasma alpha-1-antitrypsin (AAT) concentrations, which were highest in February and lowest in the summer months (Figures 5C and 6A). Thus, the periodicity of AAT is opposite to the periodicity of the cytokines that are highest in summer (displayed in Figure 6B for IL-1β after stimulation with MSU + C16 and Figure S5 for others). AAT is a known anti-inflammatory mediator (Bergin et al., 2012; Joosten et al., 2016), but to the best of

(E) Boxplot visualization of cytokine production with gender/oral contraceptive usage.
(F) Correlations between progesterone/testosterone levels and cytokine/IgA levels for men and women. Tests were performed for all immunological responses showing a significant relation to gender. Only the resulting significant correlations are shown in this plot.
(G) Scatterplot of the relation between leptin and testosterone in men, where the lines indicate the LOESS fit.
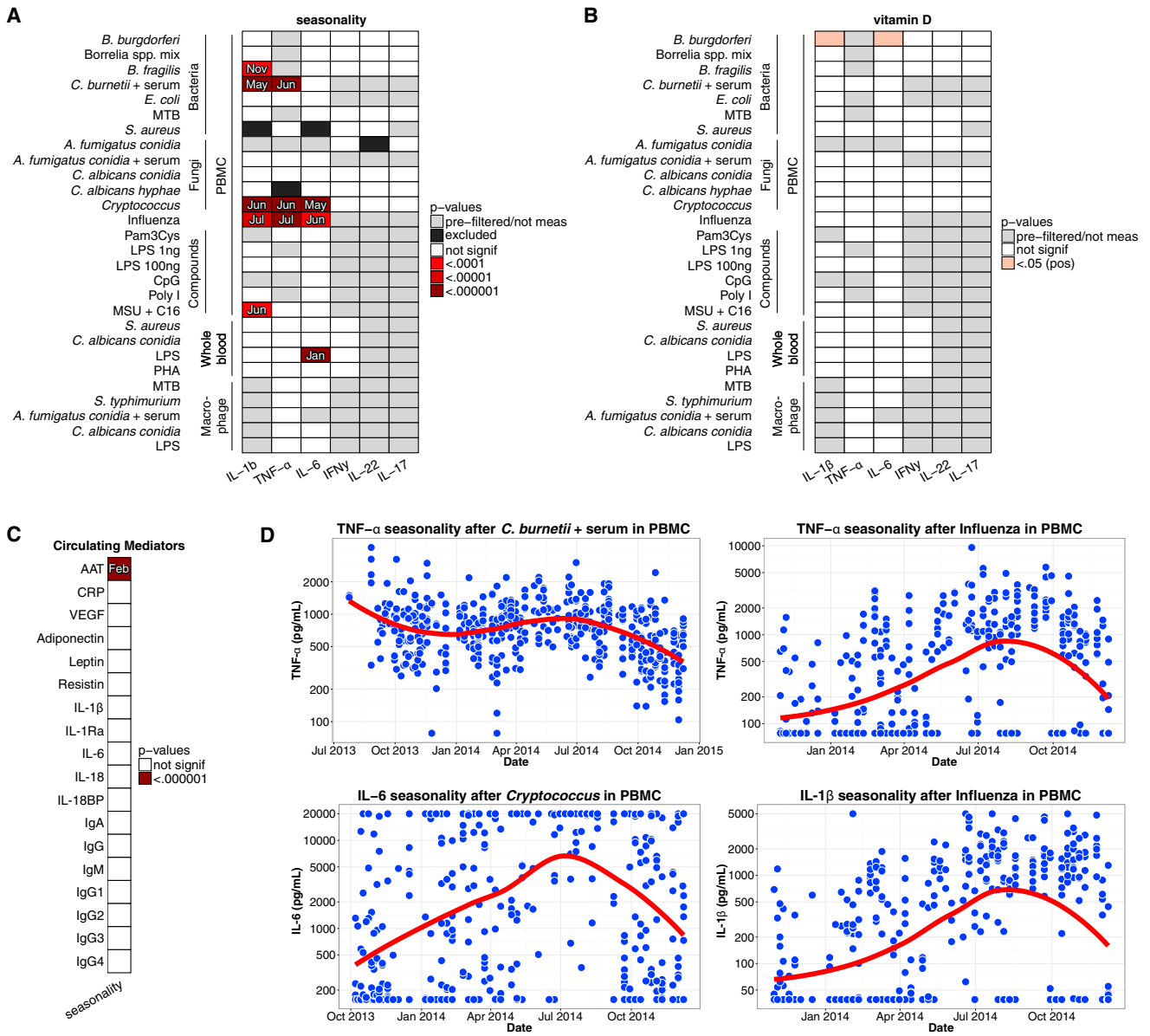See also Figures S2 and S3 and Tables S1 and S3.

Figure 5. Seasonal Changes in Cytokine Levels

(A) Heatmap showing the cytokines having seasonal responses. Three letter abbreviation indicate the month at which the production of the cytokine (x axis) is highest. A few stimulus-cytokine combinations were excluded (see legend), because the time profile showed clear storage degradation effects which interfere with the seasonality analysis.

(B) Effects of vitamin D levels shown in a heatmap similar to Figure 3A, red indicates a positive relation.

(C) Heatmap showing the significance of the seasonality of the circulating cytokines.

(D) Examples of some of the seasonal responses, where each blue dot is an individual measurement and the red line depicts the LOESS curve.

See also Figures S2, S3, S4, and S5 and Tables S3, S4, S5, S6, and S7.

our knowledge, nothing is known about its seasonal variation. Because of the opposite seasonality of AAT with cytokine induction by uric acid crystals and fatty acids (C16) (Figure 6B), we validated the impact of AAT on cytokine response in sterile inflammation in an experimental model of gout, a condition caused by the deposition of MSU crystals in the joints. When a plasma-derived form of AAT (Prolastin C) was injected in mice

challenged with uric acid crystals and fatty acids intra-articularly, joint swelling was strongly reduced (Figure 6C), the synovial IL-1β production was significantly inhibited (Figure 6D), and histology showed lower cellular infiltration in the joint cavity after AAT treatment (Figures 6F and 6G).

To demonstrate the clinical relevance of these findings, we thereafter assessed whether the seasonal periodicity of
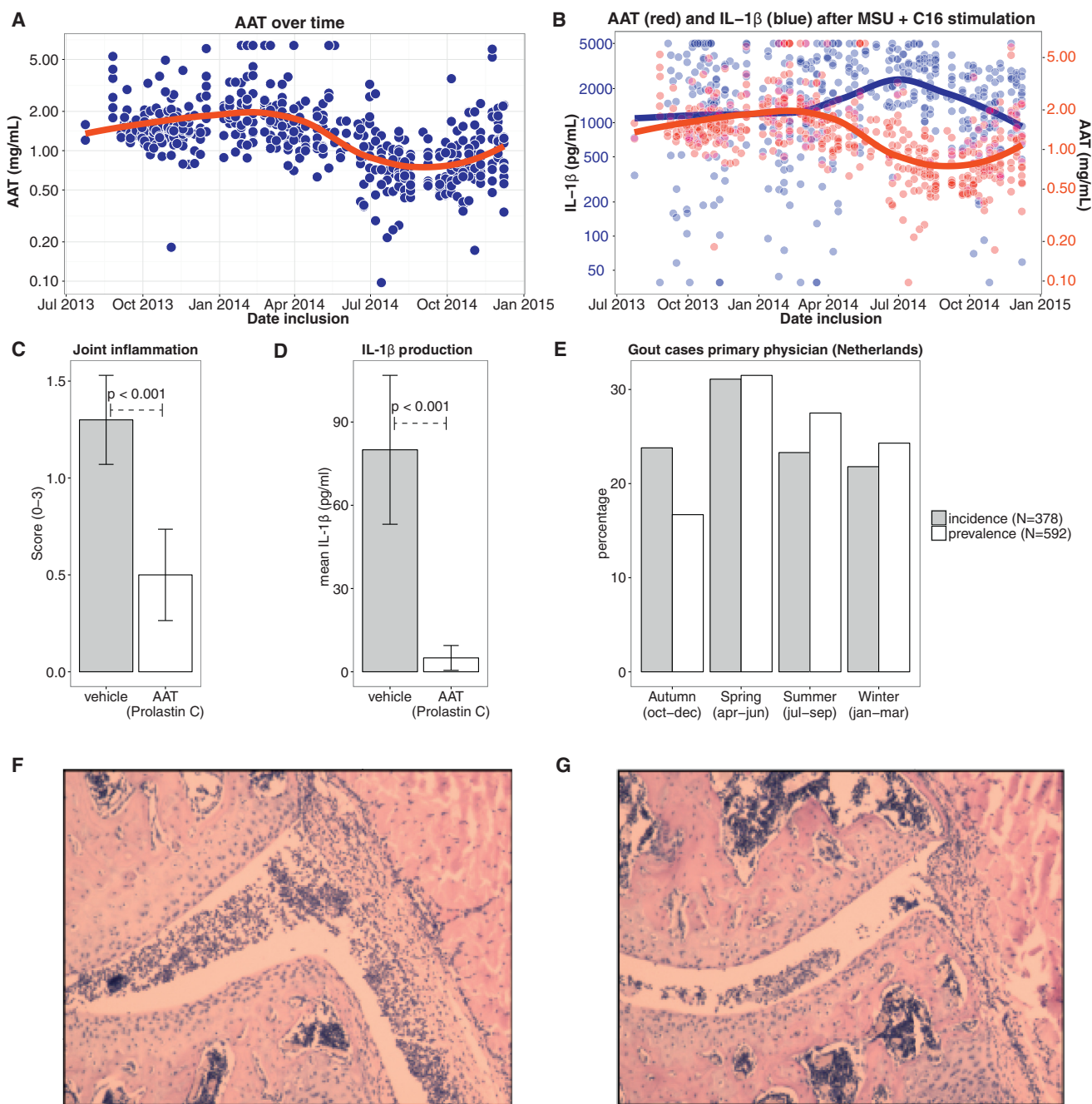
Figure 6. AAT Effects on IL-1b Production and Gout Prevalence

(A) Scatterplot of the seasonal response of AAT showing a decrease in summer. The line indicates the LOESS fit.

(B) Combined plots of AAT and IL-1b after influenza stimulation showing their opposite seasonal periodicity. The lines depict LOESS curves through the scatterplots.

(C) Bar plots showing decreased joint inflammation in mice injected with uric acid crystals after being injected AAT. Data are represented as mean ± SEM.

(D) Bar plots showing decreased IL-1β production in mice injected with uric acid crystals after being injected AAT. Data are represented as mean ± SEM.

(E) Number of patients presenting with gout at a primary physician in the Netherlands (n = ~800), with a clear increase in spring/summer.

(F and G) Histopathology of an inflamed knee joint of an vehicle (BSA, 100 μg/kg)-treated mouse, 4 hr after induction of gouty arthritis induced by intra-articular injection of MSU/C16.0 (300 μg/200 mM). Note the severe infiltration of cells in the joint cavity (F). Human plasma-derived AAT (Prolastin C, 100 μg/kg)-treated mouse, showing decreased inflammation (G). H&E staining, original magnification, 200×.

See also Figure S5 and Tables S5, S6, and S7.

MSU-induced cytokine responses may impact the clinical picture of gout. Indeed, retrospective assessment of the incidence and prevalence of inflammatory exacerbations in a cohort of more than 800 patients with gout identified a clear seasonality profile, with the peak in spring/summer when the cytokine production of IL-1β is highest and plasma AAT is the lowest level (Figure 6E). It is also important to note the highly negative correlation of plasma AAT with plasma IL-1β in the same plasma sample, which is consistent with AAT negatively regulating IL-1β production in vivo. All in all, this suggests that AAT is not only anti-inflammatory, but that it specifically decreases IL-1β production in a seasonal fashion and that this has a clear clinical relevance to at least one very important autoinflammatory disorder.

DISCUSSION

The HFGP aims to understand the individual sources of variability in immune responses by studying human cytokine production capacity in response to a comprehensive panel of microbial and metabolic stimuli. We systematically investigated the factors that influence the human cytokine responses in the 500 Functional Genomics (500FG) cohort of healthy volunteers within the HFGP, after stimulation of their leukocytes with bacterial, fungal, viral, and non-microbial metabolic stimuli; while the present study assessed the impact of environmental and non-genetic host factors on cytokine responses, complementary studies investigated the impact of the genetic (Li et al., 2016) and microbiome (Schirmer et al., 2016) variability on cytokine production.

An important conclusion of the present study is that non-genetic host factors such as age or gender have a clear effect on cytokine responses, and most of these effects are cytokine- and/or stimulus-dependent. For example, old age is associated with clear defects in the production of the T-helper cytokine products IL-22 and IFNγ, while the production of monocyte-derived cytokines and IL-17 does not change with age. Changes in immune responses due to old age are well-documented, but have been performed in smaller cohorts, resulting in conflicting data: while some have reported defective TLR-induced cytokine responses in dendritic cells of elderly individuals (Panda et al., 2010), others have not identified such effects (Janssen et al., 2015). Lymphocyte defects in the elderly have been previously described (Ferrando-Martínez et al., 2011; Swain et al., 2005), and our data present an important biological correlate to this defect. The defective adaptive lymphocyte responses may thus at least partly explain the poor response to vaccination in the elderly (Weinberger et al., 2008). In contrast, the intact innate immune responses (production of monocyte-derived cytokines) can present an opportunity to initiate a new strategy of vaccination in the elderly based on trained immunity (innate immune memory) (van der Meer et al., 2015). Additionally, the presence of higher concentrations of several circulating inflammatory mediators in the elderly (such as IL-6, IL-1Ra) may be a mirror of the low-grade inflammatory condition described as "inflammaging" (Baylis et al., 2013) and that has been hypothesized to be responsible for some of the age-related chronic diseases associated with inflammation.

Another important host factor that influences immune responses is gender (Oertelt-Prigione, 2012), resulting in a differ-ential susceptibility of men and women to infectious, auto-immune, and inflammatory diseases (Libert et al., 2010). Understanding the gender-related aspects of cytokine biology was therefore an important aim of our study. We found that monocyte-derived cytokine production was higher in men in several stimulation assays, which may contribute to the increased susceptibility of men to inflammatory diseases such as insulin resistance or atherosclerosis (Geer and Shen, 2009; Towfighi et al., 2009). In contrast, Th17 responses were higher in women using *Candida albicans* hyphae as a model stimulation system. High IL-17 production could be a driver for a higher incidence of several autoimmune diseases such as multiple sclerosis or rheumatoid arthritis in women (Gaffen, 2004; Gold and Lühder, 2008; Kotake et al., 1999; Lock et al., 2002), although this remains to be demonstrated in future studies. On the other hand, the higher *Candida*-induced IL-17 production in women may just be a mirror of more prevalent *Candida* colonization, e.g., at the level of vaginal mucosa. In contrast to age and gender, other important host-related factors such as BMI or smoking did not exert a sizeable effect on cytokine production capacity.

In addition to host factors, the immune responses of an individual are also likely to be affected by the environment, and this study aimed to comprehensively assess the role of both environmental and host factors in cytokine responses. One of the most interesting observations is the role of seasonality as a factor influencing cytokine production variability. This is biologically very relevant especially given the seasonality of many infectious diseases (Bonsall et al., 2015). A separate HFGP manuscript that assessed variation of cell counts in the 500FG cohort supports the importance of seasonality on immune parameters (Aguirre-Gamboa et al., 2016). Importantly, while previous smaller studies suggested seasonal fluctuations of vitamin D levels as the main explanation for seasonal effects (Khoo et al., 2011a, 2011b, 2012), this hypothesis is not supported by our study. The absence of direct effects on vitamin D concentrations on human cytokine responses is a crucial finding that needs further investigation: because vitamin D is seen as a possible target for health policy intervention, its true impact on biological processes in large cohorts of individuals needs to be thoroughly assessed before such policies are implemented. In contrast, our study unravels an unknown effect of seasonality on the circulating concentration of AAT, one of the most important acute phase proteins. AAT shows an inverse correlation with the production of cytokines after several stimulations, among them MSU, suggesting an anti-inflammatory effect of AAT. The biological importance of our findings is validated in an experimental model of gouty arthritis in mice (a disease caused by the formation of uric acid crystals). Even more significantly, the peak of uric acid-induced IL-1β production in summer is also validated by an increased incidence and prevalence of gout attacks during summer in a large cohort of patients. This demonstrates both the validity of the hypotheses extracted from the HFGP database, as well as the clinical impact of these processes.

All in all, the findings of this study define the main environmental and non-genetic host factors that impact immune responses. In order to comprehensively investigate the factors that influence cytokine production, complementary studies

presented in this issue of *Cell* investigate the impact of genetic variation and of the gut microbial flora on the same responses. The accompanying study by Li et al. demonstrates that genetic variability of the host has a strong effect on cytokine responses, again with the impact depending on the type of pathogen and cytokine studied (Li et al., 2016). In addition, a role of non-genetic factors for the modulation of cytokine responses is supported by the identification of important microbiome components that modulate cytokine responses, as described in the accompanying manuscript by Schirmer et al. (2016). These effects are likely mediated by microbial components or through diverse metabolites released by microbiota-diet interaction. These three complementary studies within HFGP that assess the host/environmental, genetic, and microbiome factors influencing cytokine responses provide a comprehensive picture of cytokine response variability in humans, potentially opening up new avenues for personalized medicine. Future studies and analyses of the cohorts from the HFGP will focus on the assessment of the effect of other factors (e.g., diet, metabolome), as well as on integrating these different datasets to be even more accurate in predicting and understanding the immune response against various pathogens.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and seven tables and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2016.10.018.

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AUTHOR CONTRIBUTIONS

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REFERENCES

Aguirre-Gamboa, R., Joosten, I., P.C.M., U., van der Molen, R.G., van Rijssen, E., van Cranenbroek, B., Oosting, M., Smeekens, S.P., Jaeger, M., Zorro, M., et al. (2016). Differential effects of environmental and genetic factors on T and B cell immune traits. Cell Rep. Published online November 3, 2016. http://dx.doi.org/10.1016/j.celrep.2016.10.053.

Anders, S., Pyl, P.T., and Huber, W. (2015). HTSeq–a Python framework to work with high-throughput sequencing data. Bioinformatics 31, 166–169.

Anderson, P. (2008). Post-transcriptional control of cytokine production. Nat. Immunol. 9, 353–359.

Aulock, S.V., Deininger, S., Draing, C., Gueinzius, K., Dehus, O., and Hermann, C. (2006). Gender difference in cytokine secretion on immune stimulation with LPS and LTA. J. Interferon Cytokine Res. 26, 887–892.

Baylis, D., Bartlett, D.B., Patel, H.P., and Roberts, H.C. (2013). Understanding how we age: insights into inflammaging. Longev. Healthspan 2, 8.

Behre, H.M., Simoni, M., and Nieschlag, E. (1997). Strong association between serum levels of leptin and testosterone in men. Clin. Endocrinol. (Oxf.) 47, 237–240.

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. B 57, 289–300.

Bergin, D.A., Hurley, K., McElvaney, N.G., and Reeves, E.P. (2012). Alpha-1 antitrypsin: a potent anti-inflammatory and potential novel therapeutic agent. Arch. Immunol. Ther. Exp. (Warsz.) 60, 81–97.

Bernstein, E.D., and Murasko, D.M. (1998). Effect of age on cytokine production in humans. Age (Omaha) 21, 137–151.

Berry, M.P., Graham, C.M., McNab, F.W., Xu, Z., Bloch, S.A., Oni, T., Wilkinson, K.A., Banchereau, R., Skinner, J., Wilkinson, R.J., et al. (2010). An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature 466, 973–977.

Bikle, D. (2009). Nonclassic actions of vitamin D. J. Clin. Endocrinol. Metab. 94, 26–34.

Blot, S.I., Vandewoude, K.H., Hoste, E.A., and Colardyn, F.A. (2002). Effects of nosocomial candidemia on outcomes of critically ill patients. Am. J. Med. 113, 480–485.

Bonsall, D.R., Kim, H., Tocci, C., Ndiaye, A., Petronzio, A., McKay-Corkum, G., Molyneux, P.C., Scammell, T.E., and Harrington, M.E. (2015). Suppression of locomotor activity in female C57Bl/6J mice treated with interleukin-1β: investigating a method for the study of fatigue in laboratory animals. PLoS ONE 10, e0140678.

Böttner, A., Kratzsch, J., Müller, G., Kapellen, T.M., Blüher, S., Keller, E., Blüher, M., and Kiess, W. (2004). Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels. J. Clin. Endocrinol. Metab. 89, 4053–4061.

Buchbinder, S., Kratzsch, J., Fiedler, G.M., Yar, V., Brügel, M., Leichtle, A., Weber, W., Alexander, H., Matthes, G., and Thiery, J. (2008). Body weight and oral contraceptives are the most important modulators of serum CRP levels. Scand. J. Clin. Lab. Invest. 68, 140–144.

Cassidy, J.T., Nordby, G.L., and Dodge, H.J. (1974). Biologic variation of human serum immunoglobulin concentrations: sex-age specific effects. J. Chronic Dis. 27, 507–516.

Cauci, S., Di Santolo, M., Culhane, J.F., Stel, G., Gonano, F., and Guaschino, S. (2008). Effects of third-generation oral contraceptives on high-sensitivity C-reactive protein and homocysteine in young women. Obstet. Gynecol. 111, 857–864.

Charnock-Jones, D.S., Macpherson, A.M., Archer, D.F., Leslie, S., Makkink, W.K., Sharkey, A.M., and Smith, S.K. (2000). The effect of progestins on vascular endothelial growth factor, oestrogen receptor and progesterone receptor immunoreactivity and endothelial cell density in human endometrium. Hum. Reprod. 15 (Suppl 3), 85–95.

Clark, T.A., and Hajjeh, R.A. (2002). Recent trends in the epidemiology of invasive mycoses. Curr. Opin. Infect. Dis. 15, 569–574.

Considine, R.V., Sinha, M.K., Heiman, M.L., Kriauciunas, A., Stephens, T.W., Nyce, M.R., Ohannesian, J.P., Marco, C.C., McKee, L.J., Bauer, T.L., et al. (1996). Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. 334, 292–295.

Correale, J., Ysrraelit, M.C., and Gaitán, M.I. (2009). Immunomodulatory effects of Vitamin D in multiple sclerosis. Brain 132, 1146–1160.

De Jong, S., Neeleman, M., Luykx, J.J., ten Berg, M.J., Strengman, E., Den Breeijen, H.H., Stijvers, L.C., Buizer-Voskamp, J.E., Bakker, S.C., Kahn, R.S., et al. (2014). Seasonal changes in gene expression represent cell-type composition in whole blood. Hum. Mol. Genet. 23, 2721–2728.

de Visser, K.E., Eichten, A., and Coussens, L.M. (2006). Paradoxical roles of the immune system during cancer development. Nat. Rev. Cancer 6, 24–37.

Diavatopoulos, D.A., Short, K.R., Price, J.T., Wilksch, J.J., Brown, L.E., Briles, D.E., Strugnell, R.A., and Wijburg, O.L. (2010). Influenza A virus facilitates Streptococcus pneumoniae transmission and disease. FASEB J. 24, 1789–1798.

Dinarello, C.A., Simon, A., and van der Meer, J.W. (2012). Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. Nat. Rev. Drug Discov. 11, 633–652.

Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T.R. (2013). STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29, 15–21.

Dopico, X.C., Evangelou, M., Ferreira, R.C., Guo, H., Pekalski, M.L., Smyth, D.J., Cooper, N., Burren, O.S., Fulford, A.J., Hennig, B.J., et al. (2015). Widespread seasonal gene expression reveals annual differences in human immunity and physiology. Nat. Commun. 6, 7000.

Fairfax, B.P., Humburg, P., Makino, S., Naranbhai, V., Wong, D., Lau, E., Jostins, L., Plant, K., Andrews, R., McGee, C., and Knight, J.C. (2014). Innate immune activity conditions the effect of regulatory variants upon monocyte gene expression. Science 343, 1246949.

Ferrando-Martínez, S., Ruiz-Mateos, E., Hernández, A., Gutiérrez, E., Rodríguez-Méndez, Mdel.M., Ordoñez, A., and Leal, M. (2011). Age-related deregulation of naive T cell homeostasis in elderly humans. Age (Dordr.) 33, 197–207.

Fishman, J.A. (2007). Infection in solid-organ transplant recipients. N. Engl. J. Med. 357, 2601–2614.

Fishman, J.A., and Rubin, R.H. (1998). Infection in organ-transplant recipients. N. Engl. J. Med. 338, 1741–1751.

Gaffen, S.L. (2004). Biology of recently discovered cytokines: interleukin-17–a unique inflammatory cytokine with roles in bone biology and arthritis. Arthritis Res. Ther. 6, 240–247.

Gandhi, R., Laroni, A., and Weiner, H.L. (2010). Role of the innate immune system in the pathogenesis of multiple sclerosis. J. Neuroimmunol. 221, 7–14.

Geer, E.B., and Shen, W. (2009). Gender differences in insulin resistance, body composition, and energy balance. Gend. Med. 6 (Suppl 1), 60–75.

Gold, R., and Lühder, F. (2008). Interleukin-17–extended features of a key player in multiple sclerosis. Am. J. Pathol. 172, 8–10.

Gonzalez-Quintela, A., Alende, R., Gude, F., Campos, J., Rey, J., Meijide, L.M., Fernandez-Merino, C., and Vidal, C. (2008). Serum levels of immunoglobulins (IgG, IgA, IgM) in a general adult population and their relationship with alcohol consumption, smoking and common metabolic abnormalities. Clin. Exp. Immunol. 151, 42–50.

Grandgirard, D., Gäumann, R., Coulibaly, B., Dangy, J.P., Sie, A., Junghanss, T., Schudel, H., Pluschke, G., and Leib, S.L. (2013). The causative pathogen determines the inflammatory profile in cerebrospinal fluid and outcome in patients with bacterial meningitis. Mediators Inflamm. 2013, 312476.

Hellström, L., Wahrenberg, H., Hruska, K., Reynisdottir, S., and Arner, P. (2000). Mechanisms behind gender differences in circulating leptin levels. J. Intern. Med. 247, 457–462.

Heneka, M.T., Golenbock, D.T., and Latz, E. (2015). Innate immunity in Alzheimer's disease. Nat. Immunol. 16, 229–236.

Hoffmann, E., Stech, J., Guan, Y., Webster, R.G., and Perez, D.R. (2001). Universal primer set for the full-length amplification of all influenza A viruses. Arch. Virol. *146*, 2275–2289.

Hwang, L.Y., Scott, M.E., Ma, Y., and Moscicki, A.B. (2015). Diversity of cervicovaginal cytokine response to incident chlamydia trachomatis infection among a prospective cohort of young women. Am. J. Reprod. Immunol. *74*, 228–236.

Jafarzadeh, A., Sadeghi, M., Karam, G.A., and Vazirinejad, R. (2010). Salivary IgA and IgE levels in healthy subjects: relation to age and gender. Braz. Oral Res. *24*, 21–27.

Janssen, N., Derhovanessian, E., Demuth, I., Arnaout, F., Steinhagen-Thiessen, E., and Pawelec, G. (2015). Responses of dendritic cells to TLR-4 stimulation are maintained in the elderly and resist the effects of CMV infection seen in the young. J. Gerontol. A Biol. Sci. Med. Sci. *71*, 1117–1123.

Joosten, L.A., Crişan, T.O., Azam, T., Cleophas, M.C., Koenders, M.I., van de Veerdonk, F.L., Netea, M.G., Kim, S., and Dinarello, C.A. (2016). Alpha-1-antitrypsin-Fc fusion protein ameliorates gouty arthritis by reducing release and extracellular processing of IL-1β and by the induction of endogenous IL-1Ra. Ann. Rheum. Dis. *75*, 1219–1227.

Joosten, L.A., Netea, M.G., Mylona, E., Koenders, M.I., Malireddi, R.K., Oosting, M., Stienstra, R., van de Veerdonk, F.L., Stalenhoef, A.F., Giamarellos-Bourboulis, E.J., et al. (2010). Engagement of fatty acids with Toll-like receptor 2 drives interleukin-1beta production via the ASC/caspase 1 pathway in monosodium urate monohydrate crystal-induced gouty arthritis. Arthritis Rheum. *62*, 3237–3248.

Kantor, E.D., Lampe, J.W., Kratz, M., and White, E. (2013). Lifestyle factors and inflammation: associations by body mass index. PLoS ONE *8*, e67833.

Khaodhiar, L., Ling, P.R., Blackburn, G.L., and Bistrian, B.R. (2004). Serum levels of interleukin-6 and C-reactive protein correlate with body mass index across the broad range of obesity. JPEN J. Parenter. Enteral Nutr. *28*, 410–415.

Khoo, A.L., Chai, L.Y., Koenen, H.J., Sweep, F.C., Joosten, I., Netea, M.G., and van der Ven, A.J. (2011a). Regulation of cytokine responses by seasonality of vitamin D status in healthy individuals. Clin. Exp. Immunol. *164*, 72–79.

Khoo, A.L., Joosten, I., Michels, M., Woestenenk, R., Preijers, F., He, X.H., Netea, M.G., van der Ven, A.J., and Koenen, H.J. (2011b). 1,25-Dihydroxyvitamin D3 inhibits proliferation but not the suppressive function of regulatory T cells in the absence of antigen-presenting cells. Immunology *134*, 459–468.

Khoo, A.L., Chai, L., Koenen, H., Joosten, I., Netea, M., and van der Ven, A. (2012). Translating the role of vitamin D3 in infectious diseases. Crit. Rev. Microbiol. *38*, 122–135.

Kloke, J.D., and McKean, J.W. (2012). Rfit: rank-based estimation for linear models rank-regression. R J. *4*, 57–64.

Kotake, S., Udagawa, N., Takahashi, N., Matsuzaki, K., Itoh, K., Ishiyama, S., Saito, S., Inoue, K., Kamatani, N., Gillespie, M.T., et al. (1999). IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J. Clin. Invest. *103*, 1345–1352.

Kovacic, J.C., Moreno, P., Hachinski, V., Nabel, E.G., and Fuster, V. (2011a). Cellular senescence, vascular disease, and aging: part 1 of a 2-part review. Circulation *123*, 1650–1660.

Kovacic, J.C., Moreno, P., Nabel, E.G., Hachinski, V., and Fuster, V. (2011b). Cellular senescence, vascular disease, and aging: part 2 of a 2-part review: clinical vascular disease in the elderly. Circulation *123*, 1900–1910.

Lee, M.N., Ye, C., Villani, A.C., Raj, T., Li, W., Eisenhaure, T.M., Imboywa, S.H., Chipendo, P.I., Ran, F.A., Slowikowski, K., et al. (2014). Common genetic variants modulate pathogen-sensing responses in human dendritic cells. Science *343*, 1246980.

Lewis, E.C. (2012). Expanding the clinical indications for α(1)-antitrypsin therapy. Mol. Med. *18*, 957–970.

Li, Y., Oosting, M., Smeekens, S., Jaeger, M., Aguirre-Gamboa, R., Le, K.T.T., Deelen, P., Ricaño-Ponce, I., Schoffelen, T., Jansen, A.F.M., et al. (2016). A functional genomics approach to understand variation in cytokine production in humans. Cell *167*, this issue, 1099–1110.

Libert, C., Dejager, L., and Pinheiro, I. (2010). The X chromosome in immune functions: when a chromosome makes the difference. Nat. Rev. Immunol. *10*, 594–604.

Lipsitch, M., and Viboud, C. (2009). Influenza seasonality: lifting the fog. Proc. Natl. Acad. Sci. USA *106*, 3645–3646.

Lock, C., Hermans, G., Pedotti, R., Brendolan, A., Schadt, E., Garren, H., Langer-Gould, A., Strober, S., Cannella, B., Allard, J., et al. (2002). Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat. Med. *8*, 500–508.

Lofgren, E., Fefferman, N.H., Naumov, Y.N., Gorski, J., and Naumova, E.N. (2007). Influenza seasonality: underlying causes and modeling theories. J. Virol. *81*, 5429–5436.

Macpherson, A.M., Archer, D.F., Leslie, S., Charnock-Jones, D.S., Makkink, W.K., and Smith, S.K. (1999). The effect of etonogestrel on VEGF, oestrogen and progesterone receptor immunoreactivity and endothelial cell number in human endometrium. Hum. Reprod. *14*, 3080–3087.

Martinon, F. (2010). Update on biology: uric acid and the activation of immune and inflammatory cells. Curr. Rheumatol. Rep. *12*, 135–141.

McCrea, K.A., Ensor, J.E., Nall, K., Bleecker, E.R., and Hasday, J.D. (1994). Altered cytokine regulation in the lungs of cigarette smokers. Am. J. Respir. Crit. Care Med. *150*, 696–703.

McNeil, M.M., Nash, S.L., Hajjeh, R.A., Phelan, M.A., Conn, L.A., Plikaytis, B.D., and Warnock, D.W. (2001). Trends in mortality due to invasive mycotic diseases in the United States, 1980-1997. Clin. Infect. Dis. *33*, 641–647.

Mosley, R.L., Hutter-Saunders, J.A., Stone, D.K., and Gendelman, H.E. (2012). Inflammation and adaptive immunity in Parkinson's disease. Cold Spring Harb. Perspect. Med. *2*, a009381.

Nielsen, N.O., Soborg, B., Borresen, M., Andersson, M., and Koch, A. (2013). Cytokine responses in relation to age, gender, body mass index, Mycobacterium tuberculosis infection, and otitis media among Inuit in Greenland. Am. J. Hum. Biol. *25*, 20–28.

Obiandu, C., Okerengwo, A.A., and Dapper, D.V. (2013). Levels of serum immunoglobulins in apparently healthy children and adults in Port Harcourt, Nigeria. Niger. J. Physiol. Sci. *28*, 23–27.

Oertelt-Prigione, S. (2012). The influence of sex and gender on the immune response. Autoimmun. Rev. *11*, A479–A485.

Oosting, M., Buffen, K., Cheng, S.C., Verschueren, I.C., Koentgen, F., van de Veerdonk, F.L., Netea, M.G., and Joosten, L.A. (2015). Borrelia-induced cytokine production is mediated by spleen tyrosine kinase (Syk) but is Dectin-1 and Dectin-2 independent. Cytokine *76*, 465–472.

Oosting, M., Kerstholt, M., ter Horst, R., Li, Y., Deelen, P., Smeekens, S., Jaeger, M., Lachmandas, E., Vrijmoeth, H., Lupse, M., et al. (2016). Functional and genomic architecture of Borrelia burgdorferi-induced cytokine responses in humans. Cell Host Microbe. Published online November 3, 2016. http://dx.doi.org/10.1016/j.chom.2016.10.006.

Opstad, T.B., Pettersen, A.A., Arnesen, H., and Seljeflot, I. (2011). Circulating levels of IL-18 are significantly influenced by the IL-18 +183 A/G polymorphism in coronary artery disease patients with diabetes type 2 and the metabolic syndrome: an observational study. Cardiovasc. Diabetol. *10*, 110.

Panda, A., Qian, F., Mohanty, S., van Duin, D., Newman, F.K., Zhang, L., Chen, S., Towle, V., Belshe, R.B., Fikrig, E., et al. (2010). Age-associated decrease in TLR function in primary human dendritic cells predicts influenza vaccine response. J. Immunol. *184*, 2518–2527.

Prietl, B., Treiber, G., Pieber, T.R., and Amrein, K. (2013). Vitamin D and immune function. Nutrients *5*, 2502–2521.

R Development Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Raj, T., Rothamel, K., Mostafavi, S., Ye, C., Lee, M.N., Replogle, J.M., Feng, T., Lee, M., Asinovski, N., Frohlich, I., et al. (2014). Polarization of the effects of autoimmune and neurodegenerative risk alleles in leukocytes. Science *344*, 519–523.

Rodriguez-Segade, S., Camiña, M.F., Carnero, A., Lorenzo, M.J., Alban, A., Quinteiro, C., and Lojo, S. (1996). High serum IgA concentrations in patients

with diabetes mellitus: agewise distribution and relation to chronic complications. Clin. Chem. *42*, 1064–1067.

Schirmer, M., Smeekens, S.P., Vlamakis, H., Jaeger, M., Oosting, M., Franzosa, E.A., Jansen, T., Jacobs, L., Bonder, M.J., Kurilshikov, A., et al. (2016). Linking the human gut microbiome to inflammatory cytokine production capacity. Cell *167*, this issue, 1125–1136.

Scott, M.E., Shvetsov, Y.B., Thompson, P.J., Hernandez, B.Y., Zhu, X., Wilkens, L.R., Killeen, J., Vo, D.D., Moscicki, A.B., and Goodman, M.T. (2013). Cervical cytokines and clearance of incident human papillomavirus infection: Hawaii HPV cohort study. Int. J. Cancer *133*, 1187–1196.

Siervo, M., Ruggiero, D., Sorice, R., Nutile, T., Aversano, M., Iafusco, M., Vetrano, F., Wells, J.C., Stephan, B.C., and Ciullo, M. (2012). Body mass index is directly associated with biomarkers of angiogenesis and inflammation in children and adolescents. Nutrition *28*, 262–266.

Soderberg, S., Olsson, T., Eliasson, M., Johnson, O., Brismar, K., Carlstrom, K., and Ahren, B. (2001). A strong association between biologically active testosterone and leptin in non-obese men and women is lost with increasing (central) adiposity. Int. J. Obes. Relat. Metab. Disord. *25*, 98–105.

Sopori, M. (2002). Effects of cigarette smoke on the immune system. Nat. Rev. Immunol. *2*, 372–377.

Swain, S., Clise-Dwyer, K., and Haynes, L. (2005). Homeostasis and the age-associated defect of CD4 T cells. Semin. Immunol. *17*, 370–377.

Swertz, M.A., Dijkstra, M., Adamusiak, T., van der Velde, J.K., Kanterakis, A., Roos, E.T., Lops, J., Thorisson, G.A., Arends, D., Byelas, G., et al. (2010). The MOLGENIS toolkit: rapid prototyping of biosoftware at the push of a button. BMC Bioinformatics *11* (*Suppl 12*), S12.

Szablewski, L. (2014). Role of immune system in type 1 diabetes mellitus pathogenesis. Int. Immunopharmacol. *22*, 182–191.

Towfighi, A., Zheng, L., and Ovbiagele, B. (2009). Sex-specific trends in midlife coronary heart disease risk and prevalence. Arch. Intern. Med. *169*, 1762–1766.

van der Meer, J.W., Joosten, L.A., Riksen, N., and Netea, M.G. (2015). Trained immunity: a smart way to enhance innate immune defence. Mol. Immunol. *68*, 40–44.

van Rooijen, M., Hansson, L.O., Frostegård, J., Silveira, A., Hamsten, A., and Bremme, K. (2006). Treatment with combined oral contraceptives induces a rise in serum C-reactive protein in the absence of a general inflammatory response. J. Thromb. Haemost. *4*, 77–82.

Veronica, G., and Esther, R.R. (2012). Aging, metabolic syndrome and the heart. Aging Dis. *3*, 269–279.

Weber-Mzell, D., Kotanko, P., Hauer, A.C., Goriup, U., Haas, J., Lanner, N., Erwa, W., Ahmaida, I.A., Haitchi-Petnehazy, S., Stenzel, M., et al. (2004). Gender, age and seasonal effects on IgA deficiency: a study of 7293 Caucasians. Eur. J. Clin. Invest. *34*, 224–228.

Weinberger, B., Herndler-Brandstetter, D., Schwanninger, A., Weiskopf, D., and Grubeck-Loebenstein, B. (2008). Biology of immune responses to vaccines in elderly persons. Clin. Infect. Dis. *46*, 1078–1084.

Wilkinson, M.D., Dumontier, M., Aalbersberg, I.J., Appleton, G., Axton, M., Baak, A., Blomberg, N., Boiten, J.W., da Silva Santos, L.B., Bourne, P.E., et al. (2016). The FAIR Guiding Principles for scientific data management and stewardship. Sci. Data *3*, 160018.

Ybarra, J., Lehmann, T.N., Golay, A., Juge-Aubry, C.E., Roux-Lombard, P., Dayer, J.M., and Meier, C.A. (2008). Gender-based dimorphic pattern for interleukin-1 receptor antagonist in type 2 diabetes mellitus. Diabetes Metab. *34*, 75–81.

Ye, C.J., Feng, T., Kwon, H.K., Raj, T., Wilson, M.T., Asinovski, N., McCabe, C., Lee, M.H., Frohlich, I., Paik, H.I., et al. (2014). Intersection of population variation and autoimmunity genetics in human T cell activation. Science *345*, 1254665.

Yu, H., Yuan, L., Yang, Y., Ma, S., Peng, L., Wang, Y., Zhang, C., and Li, T. (2016). Increased serum IgA concentration and plasmablast frequency in patients with age-related macular degeneration. Immunobiology *221*, 650–656.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
ProlastinC (AAT)	Grifols	N/A
BSA	SIGMA	A7030
Critical Commercial Assays		
Human IL-1β ELISA Kit	RD Systems	DY201
Human IL-6 ELISA Kit	PeliKine Compact	M9316
Human TNF-α ELISA Kit	RD Systems	DY210
Human IL-22 ELISA Kit	RD Systems	DY782
Human IL-17 ELISA Kit	RD Systems	DY317
Human IFNγ ELISA Kit	PeliKine Compact	M9333
Human AAT ELISA Kit	RD Systems	DY1268
Human Resistin ELISA Kit	RD Systems	DY1359
Human Leptin ELISA Kit	RD Systems	DY398
Human Adiponection ELISA Kit	RD Systems	DY1065
Human IL-1Ra ELISA Kit	RD Systems	DRA00B
Human IL-18Bp ELISA Kit	RD Systems	DY119
IgM,IgG,IgA	Beckman Coulter	In house
IgG subclasses	Binding Site	BN II Combi Kit
25-hydroxy vitamin D3 measurement	LCMSMS	In house
Testosterone measurement	LCMSMS	In house
Progesterone measurement	LCMSMS	In house
Plasma IL-1β	Protein Simple	Simple Plex cartridges
Plasma IL-6	Protein Simple	Simple Plex cartridges
Plasma IL-18	Protein Simple	Simple Plex cartridges
Plasma VEGF	Protein Simple	Simple Plex cartridges
Mouse IL-1β	RD Systems	DY401
TruSeq RNA sample preparation kit v2	Illumina	RS-122-2001
Deposited Data		
ELISA cytokine measurents and other cohort data:	This paper/ BBMRI-NL data infrastructure	https://hfgp.bbmri.nl/; http://www.bbmri-eric.eu/news-events/bbmri-eric-directory-2-0/
RNA sequencing of 88 individuals	This paper/ BBMRI-NL data infrastructure	https://hfgp.bbmri.nl/
Climatological data	Koninklijk Nederlands Meteorologisch Instituut	http://projects.knmi.nl/klimatologie/daggegevens/selectie.cgi
Human genome for RNaseq mapping: GRCh37.75	Ensembl	http://ftp.ensembl.org/pub/release-75/fasta/homo_sapiens/dna/
Experimental Models: Organisms/Strains		
Human PBMCs and Human Monocytes	Primary/Healthy volunteers	N/A
Mouse: C57BL/6	The Jackson Laboratory	Strain Code: 027
Sequence-Based Reagents		
Life Technologies Globin Clear kit	Illumina	www.illumina.com
Paired End cluster kit	Illumina	www.illumina.com
HiSeq2500 SBS Sequencing reagents	Illumina	www.illumina.com
Infinium CoreExome-24 v1.1 kit	Illumina	www.illumina.com

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and Algorithms		
R programming language	R Development Core Team (2015)	https://www.R-project.org/
Rfit: an R based function to perform rank based regression	Kloke and Mckean (2012)	https://cran.r-project.org/web/packages/Rfit/index.html
Custom scripts in the r programming language based on function like: lm {stats}, cor {stats}, cor.test {stats}	R Development Core Team (2015)	https://www.R-project.org/
RNA sequencing mapping: STAR (version 2.3.0)	Dobin et al. (2013)	https://github.com/alexdobin/STAR
RNA read counting: HTSeq (version 0.5.4p3)	Anders et al. (2015)	http://www-huber.embl.de/users/anders/HTSeq/doc/overview.html
Other		
See Table S1 for stimuli that have been used for in vitro stimulation experiments of PBMCs, Macrophages and Whole blood.	N/A	N/A

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for reagents may be directed to, and will be fulfilled by the Lead contact and corresponding author Mihai G. Netea at the Radboud University Medical Center, Nijmegen, the Netherlands (mihai.netea@radboudumc.nl).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Main Cohort

The 500FG cohort consists of 534 healthy individuals of Caucasian origin and is part of the Human Functional Genomics Project (HFGP). The inclusion of the volunteers took place between 8/2013 until 12/2014 in the Radboud University Medical Center, the Netherlands. Baseline characteristics of the cohort are shown in Figure 2 and Table S1. Out of the 534 individuals, 45 were excluded in the final analysis after examining the answers of their questionnaire and genetic results (Methods Details), leaving 489 individuals.

The study was approved by the Ethical Committee of Radboud University Medical Center Nijmegen (NL42561.091.12, 2012/550). Inclusion of volunteers and experiments were conducted according to the principles expressed in the Declaration of Helsinki. All volunteers gave written informed consent before any material was taken.

Validation Cohorts
Gout Patient Cohorts

Patients from 7 general practices (from 1971-2002), 3 practices from general health centers (1994-2002) as well as Dutch institute of primary care (2001-2002) were included in this validation study. Information about gout attacks and morbidity was assessed using available databases provided by these institutions. Prior to the statistical analysis we categorized the seasons into: 1/1-31/3 (winter); 1/4-30/6 (spring); 1/7-30/9; (summer); 1/10-31/12 (autumn).
300 Functional Genomics (300FG) Cohort

As a part of the HFGP a cohort of 300 individuals with a BMI higher than 27 was also recruited. Out of these 300 individuals, a sub-cohort of individuals with no diagnosed health problem was selected ($N\sim100$). For these individuals circulating IL-6 levels were measured, and these levels were used to validate the relation between age and BMI with IL-6. The study was approved by the Ethical Committee of Radboud University Medical Center Nijmegen (N146846.091.13, 2013/505). Inclusion of volunteers and experiments were conducted according to the principles expressed in the Declaration of Helsinki. All volunteers gave written informed consent before any material was taken.

Animal Model of Gouty Arthritis

Joint inflammation was induced by intra-articular injection (i.a.) MSU crystals mixed with C16.0 into the right knee joint of naive male C57BL/6 mice. The mice were between 20-25 g, housed in standard housing, 5 mice per cage. All procedures performed were in accordance with the ethical standards of, and approved by, the Ethical review board of University of Colorado Denver, USA.

METHODS DETAILS

Demographic Data Collection

After visiting the hospital to donate blood, the volunteers received an extensive online questionnaire about lifestyle, diet, and disease history. Based on the results of this questionnaire we had to exclude 45 volunteers for various reasons, e.g., they were under medication, non-European ancestry, or had a chronic disease. By excluding these individuals from the analysis we minimized false positive effects on the cytokine production capacity in vitro and in vivo.

In Vitro Cytokine Stimulation Assays in the Three Systems

After obtaining informed consent, venous blood was drawn from a cubital vein of volunteers into sterile 10 mL EDTA tubes, 8 mL serum tubes as well as 8ml heparine tubes (Monoject).

PBMC Stimulation Experiments

Isolation of PBMCs was performed as described in Oosting et al. (2015). Cells were washed twice in saline and suspended in medium (RPMI 1640) supplemented with gentamicin 10 mg/mL, L-glutamine 10 mM and pyruvate 10 mM. PBMC stimulations were performed with 5×10^5 cells/well in round-bottom 96-wells plates (Greiner) for either 24 hr or 7 days in the presence of 10% human pool serum at 37°C and 5% CO_2. Additional details are available in the STAR Methods. Supernatants were collected and stored in −20°C until used for ELISA. The stimulations used for the 24 hr and 7 day experiments are shown in Table S1.

Macrophage Stimulation Experiments

In order to perform macrophage stimulation experiments we plated 5×10^5 cells in flat-bottom plates (Greiner) with 10% human serum at 37°C and 5% CO_2 which was refreshed after 3 days. After another 3 days the medium was removed and cells adherent macrophages were stimulated for 24 hr. Supernatants were collected and stored in −20°C until used for ELISA. The stimulations used for the 24 hr macrophage experiment are shown in Table S1.

Whole Blood Stimulation Experiments

100 μL of heparin blood was added to a 48 well plate and subsequently stimulated with 400ul stimulus (final volume 500ul) for 48 hr at 37°C and 5% CO_2. Supernatants were collected and stored in −20°C until used for ELISA. The stimulations used for the 48 hr whole blood experiment are shown in Table S1.

Influenza Culture en Inactivation

Influenza virus strain pH1N1 A/Netherlands/602/09 (kindly provided by Prof. Ron Fouchier, Erasmus MC) was grown in the allantoic fluid of embryonated chicken eggs as described previously (Diavatopoulos et al., 2010). Viral titers were determined by three independent plaque assays performed on Madin-Darby canine kidney (MDCK) cells (Hoffmann et al., 2001). To inactivate the pH1N1 strain, β-propiolactone (BPL) (Acros Organics, Morris Plans) in citrate buffer (125 mM sodium citrate, 150 mM sodium chloride [pH 8.2]) was added to the pH1N1 virus to a final concentration of 0.1% and incubated for 24h at 4°C under continuous slow shaking. Inactivated virus was subsequently snap-frozen and stored at −80°C. Virus inactivation was confirmed by three passages in MDCKs where no virus could be detected by plaque assay following the third passage.

ELISA Analysis

Samples of all experiments were measured at once using the following ELISA kits:

In the 24 hr PBMC stimulation experiments we measured concentrations of human IL-1β, IL-6 as well as TNF-α (PeliKine Compact or R&D Systems). Supernatants of the 7 days stimulation assays were used to measure IL-22, IL-17 or IFN-γ (PeliKine Compact or R&D Systems). For the whole blood samples IL-6, TNF-α, IL-1β as well as IFN-γ levels were determined (PeliKine Compact or R&D Systems). Supernatants from the macrophage stimulation experiment were used for IL-6 and TNF-α measurements (PeliKine Compact or R&D Systems).

Measurements of Circulating Mediators

The circulating mediators resistin, leptin, adiponectin, CRP and alpha-1 antitrypsin (AAT) were measured in EDTA plasma using the R&D Systems DuoSet ELISA kits following the Manufacturer's protocol. The plasma cytokines IL-1Ra and IL-18 binding protein (IL-18BP) were measured using R&D Quantikine kits following the manufacturer's standard protocol. Plasma IL-1β, IL-6, IL-18 and VEGF were measure in Simple Plex cartridges using the Ella apparatus (Protein Simple, San Jose). The IL-6 levels for the 300FG validation cohort were measured similarly. The data generated using Simple Plex cartridges correlated with physiological, environmental, and other circulating factors. Resting mean level of IL-1β in the cohort was 0.33 ± 0.37 pg/mL (range 0.04-11 pg/mL), mean total IL-18 was 163 ± 3.5 pg/mL (range 0.4-838), mean AAT levels were 1.54 ± 1.16 mg/mL and in the peak season (Feb) levels were 2.93 ± 1.81 mg/mL, mean VEGF was 35.91 ± 1.04 (range 1.15-186) and mean IL-6, 1.25 ± 0.06 pg/mL (range 0.15-8.1). These levels IL-6 levels correlated with age, BMI, CRP, IL-1β and IL-1Ra, total IL-18 and VEGF (see Figures 3A and 3C).

Vitamin D Measurements

25-hydroxy vitamin D3 (25OH-vitamin D3) was analyzed by LCMSMS after protein precipitation and solid-phase extraction. Internal standard [²H3] 25OH-vitamin D3 (Bioconnect) was added to 100 uL serum. 50 µL NaOH (2M) was added to release protein-bound 25OH-vitamin D3 and subsequently 500 µL Acetonitrile/Methanol (9:1) was added for protein precipitation. 700 µL H2O was added to 400 µL supernatant followed by solid phase extraction (Oasis HLB 1cc, Waters). Columns were conditioned with 1 mL methanol/isopropanol (95:5) and subsequently washed with 1 mL H2O. After application of the sample, columns were washed with 1 mL H2O and 1 mL methanol/H2O (60:40). The eluate (300 µL methanol/isopropanol 95:5) was diluted with H2O (3:1) and injected (10 µL) into an Agilent Technologies 1290 Infinity VL UHPLC-system (Agilent Technologies, Santa Clara, CA), equipped with a BEH C18 (1.7 µm 2.1 × 50mm) analytical column (Waters) at 45°C. Mobile phase A (methanol:water 20:80 + 2 mM NH4CH3COO + 0.1% formic acid) and B (methanol:water 98:2 + 2 mM NH4CH3COO + 0.1% formic acid) were run in a gradient (0.4 mL/min). The gradient program was as follows: Start gradient 30:70 A:B to 5:95 A:B in 3.5 min and return to 30:70 A:B in 0.5 min. Retention time was 2.73 min, total run time was 4 min. An Agilent 6490 tandem mass spectrometer (Agilent Technologies) was operated in the electrospray positive ion mode, with a capillary voltage 3.5 kV, fragmentor voltage 380 V, sheath gas temperature 350°C and gas temperature 100°C with N_2 collision gas. Both 25OH-vitamin D3 and 25OH-vitamin D3 [-H2O] (in-source fragmentation) were used for quantification (results were averaged) with both two transitions (qualitative and quantitative) monitored. Transitions (Q1 > Q3) were m/z 401.4 > 159.1 (27 kEV) and m/z 401.4 > 107.1 (27 kEV) for 25OH-vitamin D3; m/z 404.4 > 109.1 (27 kEV) and m/z 404.4 > 162.1(30 kEV) for [²H3] 25OH-vitamin D3; m/z 383.4 > 107.1 (36 kEV) and m/z 383.4 > 257.2 (16 kEV) for 25OH-vitamin D3 [-H2O]; m/z 386.4 > 109.1 (27 kEV) and m/z 386.4 > 162.1 (27 kEV) for [²H3] 25OH-vitamin D3 [-H2O]. Dwell time 25 ms. An 8-point calibration curve was used and absolute concentration of the calibrator (Sigma-Aldrich) was assessed by spectrophotometry (264nm). The method was linear assessed by CLSI EP6 protocol. Recovery was within 90%– 109%. Within-run and between-run CV is 6.4% and 6.1% at 23 nmol/L) and 5.1% and 5.5% at 81 nmol/L as assessed by adapted CLSI EP5 protocol. LOQ was 7 nM (10% CV).

Immunoglobulin Measurements

Serum levels of IgG, IgM and IgA were determined by immunonephelometry using a Beckman Coulter Immage (Beckman Coulter, Fullerton) and Beckman Coulter reagents. Measurements were standardized using certified european reference material 470 (ERM-DA470). Reference values for serum Ig are: total IgG 7.0 −16 g/l, IgM 0.4-2.3g/l and IgA 0.7-4.0g/l.

IgG subclass measurements in serum were performed on a BN II immunonephelometer (Siemens Healthcare, Erlangen, Germany) using the Binding Site (Birmingham) Human IgG Subklass BN II Combi Kit. Values were standardized using the N protein standard SL (OQIM, Siemens Healthcare), which is based on the Sanquin (Amsterdam) nephelometric standard M1590. Reference values are: IgG1 4.9 – 11.4g/l, IgG2 1.5-6.4g/l, IgG3 0.2-1.1g/l and IgG4 0.08-1.4g/l.

Hormone Measurements

Testosterone and progesterone were analyzed by LCMSMS after protein precipitation and solid-phase extraction. Internal standard [¹³C3]-testosterone (Isoscience, King of Prussia, PA) and [²H9]-progesterone (CDN isotopes) was added to 100 µL serum. Subsequently 300 µL Acetonitrile + 0.1% formic acid was added for protein precipitation. 300 µL H2O was added to 200 µL supernatant followed by solid phase extraction (Oasis HLB 1cc, Waters). Columns were pre-equilibrated with 1 mL methanol/isopropanol (95:5) and subsequently washed with 1 mL H2O. After application of the sample, columns were washed with 1 mL H2O and 1 mL methanol/H2O (30:70). The 300 µL eluate (methanol/isopropanol 95:5) was dried under a stream of N_2 gas, reconstituted in methanol: water (30:70) and injected (10 µL) into an Agilent Technologies 1290 Infinity VL UHPLC-system (Agilent Technologies, Santa Clara, CA) equipped with a BEH C18 (1.7 µm 2.1 × 50mm) analytical column (Waters) at 60°C. Mobile phase A (methanol:water 20:80 + 2 mM NH4CH3COO + 0.1% formic acid) and B (methanol:water 98:2 + 2 mM NH4CH3COO + 0.1% formic acid) were run in a gradient (0.4 mL/min). The gradient program was as follows: Start gradient 70:30 A:B for 2.5 min; then to 40:60 A:B in 3.5 min; followed by a gradient in 0.5 min to 2:98 to remain such for 0.5 min and thereafter to 70:30 A:B in 0.5 min and remain such for 0.5 min. Retention time was 4.37 min and 6.04 min for testosterone and progesterone respectively. Total run time was 8 min. An 9-point calibration curve was used (testosterone (Steraloids); progesterone (Sigma)). An Agilent 6490 tandem mass spectrometer (Agilent Technologies) was operated in the electrospray positive ion mode, with a capillary voltage 3.5 kV, fragmentor voltage 380 V, sheath gas temperature 350°C and gas temperature 150°C with N_2 collision gas. Two transitions (qualitative and quantitative) were monitored. Transitions (Q1 > Q3) were m/z 289.2 > 109.1 (30 kEV) and m/z 289.2 > 97.1 (30 kEV) for testosterone; m/z 292.3 > 112.1 (30 kEV) and m/z 292.3 > 100.1(30 kEV) for [¹³C3]-testosterone; m/z 315.3 > 109.1 (29 kEV) and m/z 315.3 > 97.1 (29 kEV) for progesterone; m/z 324.3 > 113.1 (29 kEV) and m/z 324.3 > 100.1 (29 kEV) for [²H9]-progesterone. Dwell time was 50 ms and 100 ms for testosterone and progesterone respectively. The method was linear assessed by CLSI EP6 protocol. Recovery was within 98.4%–103% (testosterone) and 99.8%–102% (progesterone). For testosterone within-run and between-run CV is 4.1% and 6.0% at 0.9 nmol/L and 3.3% and 5.3 at 19 nmol/L as assessed by adapted CLSI EP5 protocol. For progesterone within-run and between-run CV is 2.8% and 5.1% at 4.9 nmol/L and 3.4% and 6.1 at 28 nmol/L as assessed by adapted CLSI EP5 protocol. LOQ was 0.05 nmol/L and 0.25 nmol/ L (15% CV) for testosterone and progesterone respectively.

Animal Model of Gouty Arthritis

Joint inflammation was induced by intra-articular injection (i.a.) of 300 μg highly pure MSU crystals mixed with 200 μM C16.0 in 10 μL of PBS into the right knee joint of naive C57BL/6 mice. Four hours after i.a. injection, joint swelling was determined, synovial tissue was isolated and knee joints were removed for histology. The mice were pre-treated with plasma derived AAT (Prolastin C) in a dose of 4 mg per mouse or BSA (vehicle) 2 hr before gouty arthritis was induced (n = 10 mice per group). Joint inflammation was measured by macroscopic scoring (Joosten et al., 2010), 0 = no swelling, 1 = mild swelling, 2 = moderated and 3 = severe swelling. After the skin was removed the joint swelling was scored, 0 = no swelling, 1 = mild swelling, 2 = moderated and 3 = severe swelling. All values exceeding 0.25 were considered joint swelling. Joint swelling scoring was performed without knowledge of the experimental groups. For total IL-1β levels, patellae with surrounding tissue were directly transferred to 200 μL 0.5% Triton X-100 in PBS. After repeated freeze-thawing IL-1β was determined by ELISA (R&D systems).

RNA Sequencing

RNA was extracted from Paxgene tubes and RNA quantity and quality were checked on a Bioanalyzer. Total RNA from whole blood was deprived of globin using Life Technologies Globin Clear kit. RNaseq libraries were prepared from 1 μg RNA of each cell population using the TruSeq RNA sample preparation kit v2 (Illumina) according to the manufacturer's instructions, and these libraries were subsequently sequenced on a HiSeq 2500 sequencer (Illumina) using paired-end sequencing of 2 × 50 bp, upon pooling of 10 samples per lane.

Environmental Parameters

Pollen counts were performed at the Elkerliek Hospital, Helmond, the Netherlands. Pollen were collected using a Burkard pollen sampler (http://www.burkardscientific.co.uk/) and were counted using a microscope as number of pollen from a certain species per m3 of air. All counts were performed by a trained technician.

Daily levels of atmospheric NH_3, O_2, SO_2, NO, NO_2 and CO were obtained from the National Institute for Public Health and the Environment (RIVM), Ministry of Health, Welfare and Sport (http://www.lml.rivm.nl/gevalideerd/).

Climate data (temperature, humidity, sunshine duration) were obtained from The Royal Netherlands Meteorological Institute (KNMI) (http://projects.knmi.nl/klimatologie/daggegevens/selectie.cgi).

QUANTIFICATION AND STATISTICAL ANALYSIS

Data Pretreatment

All cytokine and circulating mediator data were log-transformed. In the cases where the response fell outside of the range of the ELISA measurement, the threshold value of the ELISA plate was used as the measured value. The rank-based regression method used for most analysis should not be affected by the non-normality of some of the data (and is unaffected by the log-transformation).

Measurements for which one of the detection limits contained > 50% of the measurements were filtered out of any further analysis. Missing values were handled on a pairwise basis (for each comparison separately): given the limited number of missing values and large number of people in our study, this was found preferable over imputing the data.

Circulating Cytokine Correlations

For the circulating cytokine correlations the linear Pearson correlation was used as implemented in the "stats" package of the programming language "R."

Statistical regression analysis

Rank based regression results (for all regression analysis except for the seasonality) were obtained using "Rfit" (Kloke and McKean, 2012). With this method, a regression value and standard error are calculated for each factor in the model, and these are used to calculate a p value. The t-statistic is defined as the ratio of these two values and a p value for each vector is calculated based on a t distribution with n-p-1 degrees of freedom where p is the number of regression parameters. The following regression formula was used (including an offset term):

$$concentration \sim age + gender + BMI + smoking + vitD + oralContraceptive + \sin\left(\frac{2*pi* numDaysFromJan2013}{366}\right)$$
$$+ \cos\left(\frac{2*pi* numDaysFromJan2013}{366}\right) + numDaysFromJan2013$$

where *sin(2 * pi * numDaysFromJan2013 / 366)* and *cos(2 * pi * numDaysFromJan2013 / 366)* are periodic signals together capturing seasonality patterns with a periodicity of one year. The linear term *numDaysFromJan2013*, indicating after how many days after Jan 1st 2013 the sample was collected, was added to partially correct for sample storage degradation. In this analysis these terms are added as correction factors, calculation of the significance of the seasonality is described below. The other factors were included as follows: *age* was added to the model as numerical (float) values, *gender* was added as a categorical value, with categories 'male' and 'female', *BMI* was added to the model as numerical (float) values, *smoking* was added

as a binary value to indicate if somebody smokes (on a regular basis), *vitD* was added as a numerical (float) value, indicating someone's vitamin D levels and *oralContraceptive* was added as a binary factor, indicating oral contraceptive usage.

Seasonality Analysis

Seasonality analysis was performed using a linear regression analysis. A linear combination of a sine and cosine term with the same frequency allows for the formation of a sine wave with any phase of that particular frequency.

This property can be mathematically described as:

$$A' \sin(\omega t) + A'' \cos(\omega t) = A \sin(\omega t + \varphi) \tag{2}$$

where

$$A = \sqrt{A'^2 + A''^2} \tag{3}$$

and

$$\varphi = tan^{-1}\left(\frac{A''}{A'}\right) \tag{4}$$

(for proof see: http://dspguru.com/sites/dspguru/files/Sum_of_Two_Sinusoids.pdf, http://math.stackexchange.com/questions/535600/sum-of-sinusoids-with-same-frequency-sinusoid-proof).

We used a sine and cosine wave with a period of one year. By fitting a linear model to each cytokine/measurement using the "lm" function part of the "stats" package in R, it is possible to estimate the amplitude and phase of a periodic signal present in the data. To achieve this we use the same regression formula (1) as was used for the rank-based model. By comparing the regression analysis with the cosine and sine term to an alternative regression model lacking these terms we obtained p values for the seasonality. The comparison between the two models was performed using analysis of variance ("anova" function in R).

Seasonality Filtering

The seasonality analysis tends to pick up spurious effects caused by sample storage (samples stored in the freezer tend to degrade over). To remove these false positives, the results were filtered on a stricter p value threshold than the other factors (p $\leq$ 0.0001) and a threshold was set on the (relative) amplitude of the seasonal term.

The amplitude of the seasonality term was calculated as defined in (3). To normalize this amplitude to the range of values for a measurement, the 10th (perc10) and 90th (perc90) percentile for each measurement were calculated. The amplitude was subsequently normalized as:

$$A_{norm} = \frac{A}{perc90 - perc10} \tag{5}$$

A threshold for the seasonality was determined by visually inspecting the histogram of Anorm (Figure S2F). Most amplitudes fall within a range of about 0 to 0.14. The cut-off threshold was placed at the first peak in the histogram after this range, at a value of 0.1601.

Non-linear Seasonality Analysis

To verify if the seasonality results were independent of the type of test used, a non-linear curve-fitting approach was also performed using the 'nls' function from R package 'stats' with algorithm 'port'. Briefly, a non-linear least-square regression model was fitted to cytokine response and circulating mediator data as a function of time of sampling, according to the following formula:

$$y \sim Amplitude * cos(2 * \pi * (t - Phase)/366) + linearIncrease * t + y_0 \tag{6}$$

where

- 'y' was the measured intensity value
- 't' was the number of days since 1st of January 2013
- 'Phase' was the day of the year at which the intensity peaked
- 'linearIncrease' was the linear increase in intensity over time
- and 'y$_0$' was the intercept (estimated average intensity on 1st of January 2013)

Vitamin D Analysis

To check if vitamin D levels held any predictive power beyond their periodic signal, the levels were separated into a periodic signal and residuals. The periodic signal with 7 degrees of freedom was fitted using the "smooth.spline" function part of the "stats" package in R. The residuals were calculated by substracting the periodic signal from the vitamin D levels.

Next, a rank-based regression model was fitted using Rfit (similar to before):

$$concentration \sim age + gender + BMI + smoking + oralContraceptive + numDaysFromJan2013 + vitD_{periodic} + vitD_{residuals}$$

This provides p values for the significance of both the periodic and the residual signal.

RNA Expression Analysis

Sequencing reads were mapped to the human genome using STAR (version 2.3.0) (Dobin et al., 2013). The aligner was provided with a file containing junctions from Ensembl GRCh37.75. Htseq-count of the Python package HTSeq (version 0.5.4p3) was used (The HTSeq package, http://www-huber.embl.de/users/anders/HTSeq/doc/overview.html) to quantify the read counts per gene based on annotation version GRCh37.75, using the default union-counting mode. Reads Per Kilobase of transcript per Million mapped reads (RPKM) values were then calculated to quantify gene expression levels.

RNA Seasonality Analysis

Seasonality analysis on the mRNA data was applied to a select subset of genes of interest to retain as much statistical power as possible. Two subsets were created: one first set of the four cytokines/molecules showing a clear pattern of seasonality at the protein expression level: TNF-α, IL-1β, IL-6 and AAT; second, a set of genes that encode for receptors of the microbial ligands and the signaling molecules inducing cytokines, assembled based on expert knowledge integrated with information obtained from various public resources, such as HGNC (http://www.genenames.org), KEGG Pathways and GO (PRRs, adaptor molecules, transcription factors). These lists are provided in Table S5 and Table S6, respectively.

The RNA seasonality analysis was performed in a similar fashion to the linear seasonality analysis described previously. Formula (1) was applied, except for the removal of the "vitD"-term, since our previous analysis showed that vitamin D had no residual influence on seasonal cytokine responses and might interfere with the seasonal (sine and cosine) terms.

Combining the Rank Based Regression and Seasonality Analysis

The p values for all measured parameters for the factors "age," "gender," "BMI," "smoking," "vitD" and "oralContraceptive" obtained using "Rfit" were merged with the p values for the "seasonality" term obtained with the 'lm' function. Multiple testing correction as described in the section "Multiple Testing Correction" was performed over all these p values simultaneously.

Hormone Correlation Analysis

For those cytokines found to be affected by gender, the influence of hormones on this effect was evaluated. The (rank-based) Spearman correlation was calculated between hormone levels and cytokines, and all p values were corrected for multiple testing (see section "Multiple testing correction).

Multiple-Testing Correction

Multiple-testing correction was applied using the Benjamini-Hochberg FDR correction (Benjamini and Hochberg, 1995) over the three large-scale analyses separately: the correlation between circulating cytokines, the main regression analysis (factors with circulating cytokines/circulating mediators and cytokines after stimulation) and the hormone correlation analysis.
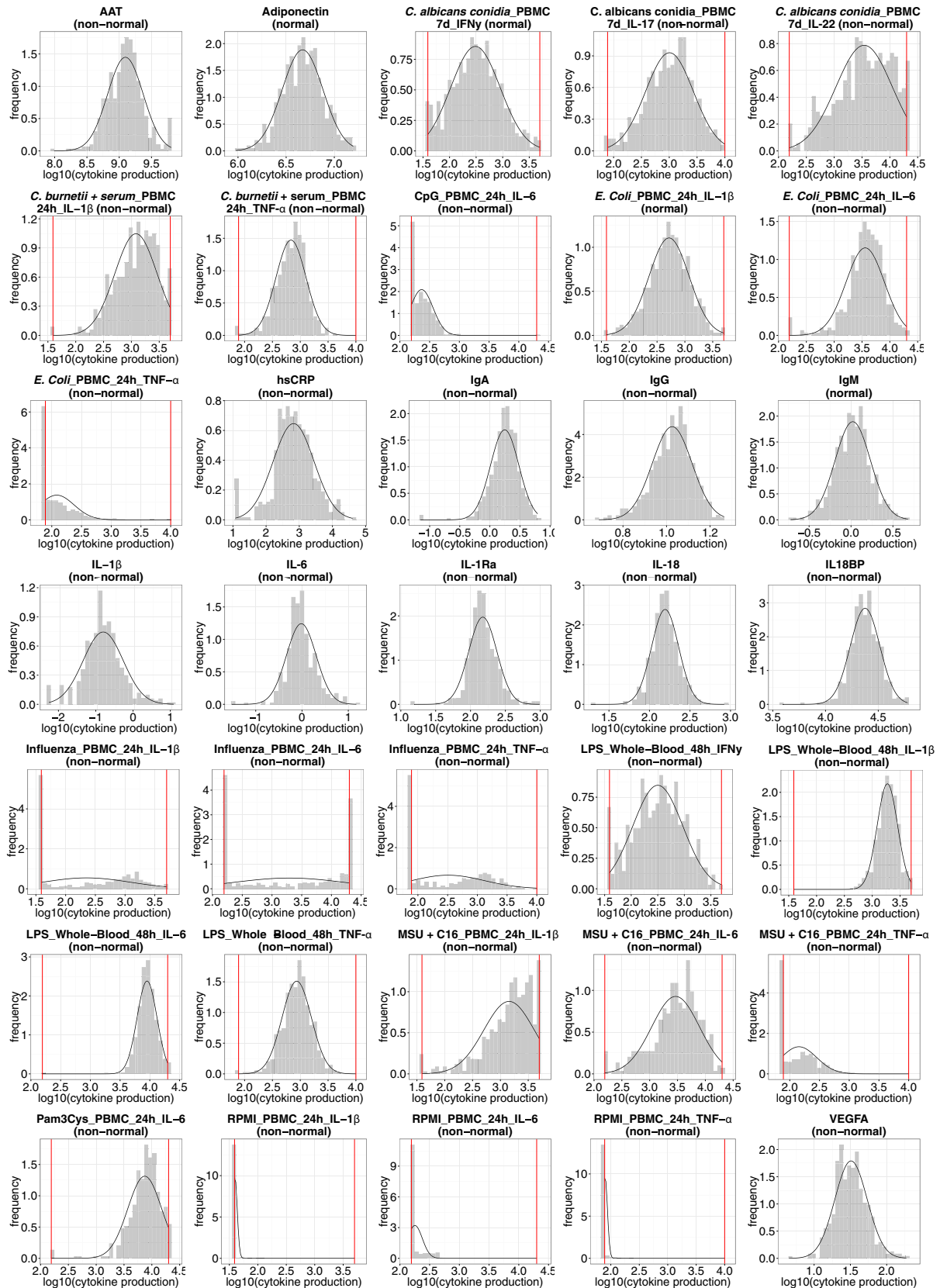
IL-6 Validation Cohort Analysis

A similar model as used in our main cohort was applied to our validation cohort using Rfit. We assessed the influence of age and gender on circulating IL-6 concentrations using the formula:

$$\begin{aligned}
concentration \sim\ & age + gender + BMI + \sin\left(\frac{2*pi*numDaysFromJan1st}{366}\right) \\
& + \cos\left(\frac{2*pi*numDaysFromJan1st}{366}\right) + numDaysFromJan1st
\end{aligned}$$

(8)

DATA AND SOFTWARE AVAILABILITY

All data used in this project have been meticulously cataloged and archived at BBMRI-NL data infrastructure at https://hfgp.bbmri.nl/ aiming for maximum reuse following the FAIR principles, i.e., Findability, Accessibility, Interoperability, and Reusability (Wilkinson et al., 2016). The central data stewardship and access has been implemented using MOLGENIS open source platform for scientific data (Swertz et al., 2010) that enables flexible data upload, management and querying, including sufficiently rich metadata and interfaces for machine processing and custom (R statistics) visualization for human processing (see http://molgenis.org). Also summaries of the study have been submitted to BBMRI central catalogs https://catalogue.bbmri.nl (Netherlands) and http://www.bbmri-eric.eu/news-events/bbmri-eric-directory-2-0/ (EU).

Figure S1. Collection of Plots Showing Several Distributions of Cytokine Expression and Circulating Mediator Concentrations, Related to Figures 1 and 2

All values were log10 transformed, as depicted on the x axis. If cytokine concentrations were measured after stimulation, the title indicates: which stimulation, in what sample type and at what time point. Also, for these samples the detection limits of the ELISA are indicated with red vertical lines. Normality of the distribution was tested using Anderson-Darling test for normality with a cut-off of $p \leq 0.05$. All concentrations were measured in pg/ml.
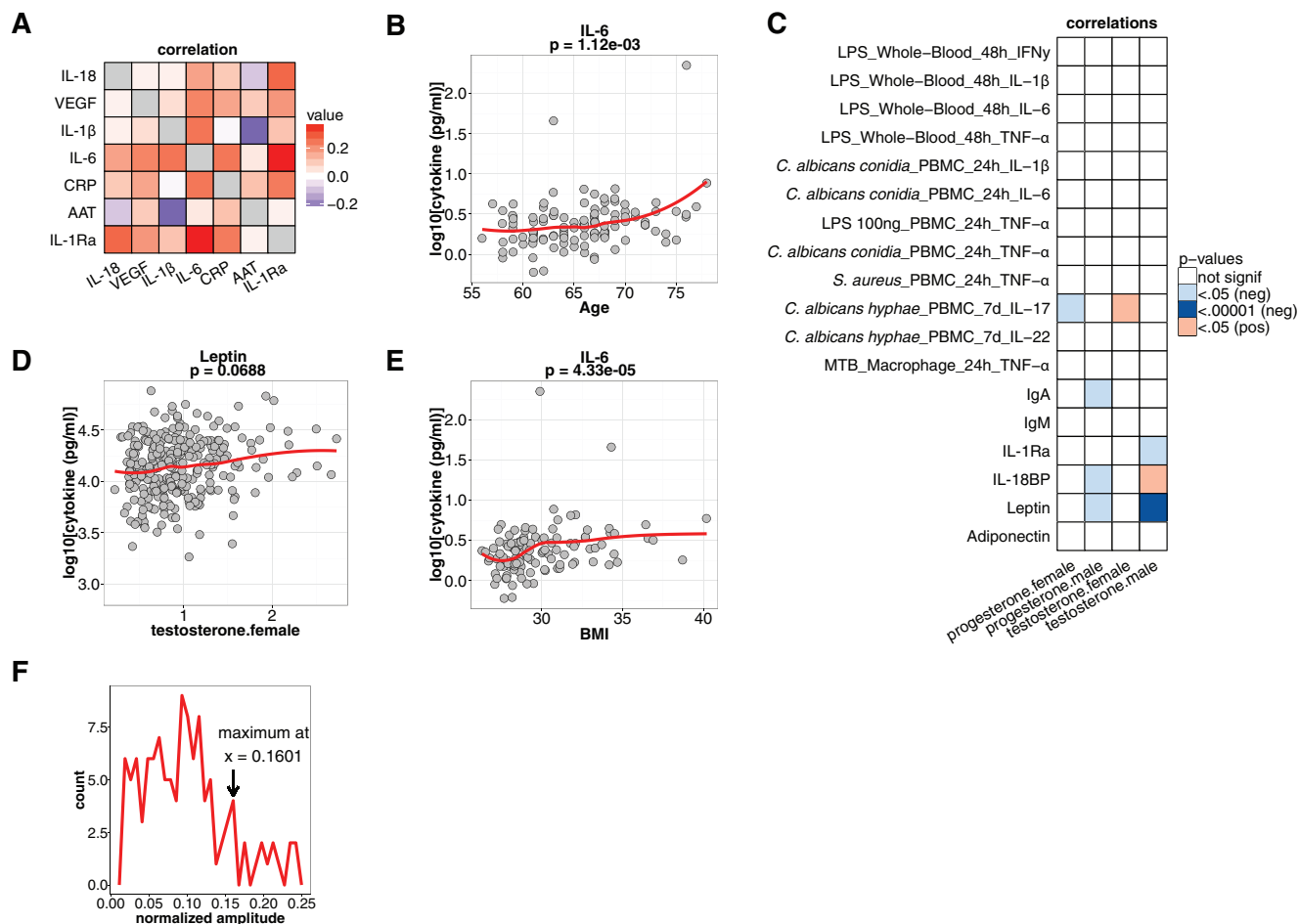
Figure S2. Plots Showing the Effect of Different Parameters on Cytokine Production and Circulating Mediators, Related to Figures 3, 4, and 5

(A) Heatmap showing the Pearson correlations between circulating cytokines.

(B) Scatterplot validating the influence of age on IL-6 levels in a healthy subset of about 100 individuals in another cohort of the Human Functional Genomics Project (HPGP). The line shows the LOESS fit to the data.

(C) Correlations between the hormones progesterone and testosterone and immunological parameters, performed separately for men and women. Tests were performed for all immunological responses showing a significant relation to gender.

(D) Scatterplot showing the relation between testosterone in men and leptin concentrations. Line shows the LOESS fit to the data.

(E) Scatterplot validating the influence of BMI on IL-6 levels in the same cohort as in A. Line shows the LOESS fit to the data.

(F) Graph showing the distribution of normalized amplitudes of the seasonality signal for all cytokines. The arrow shows the cut-off value below which the amplitude of seasonality was not considered significant.
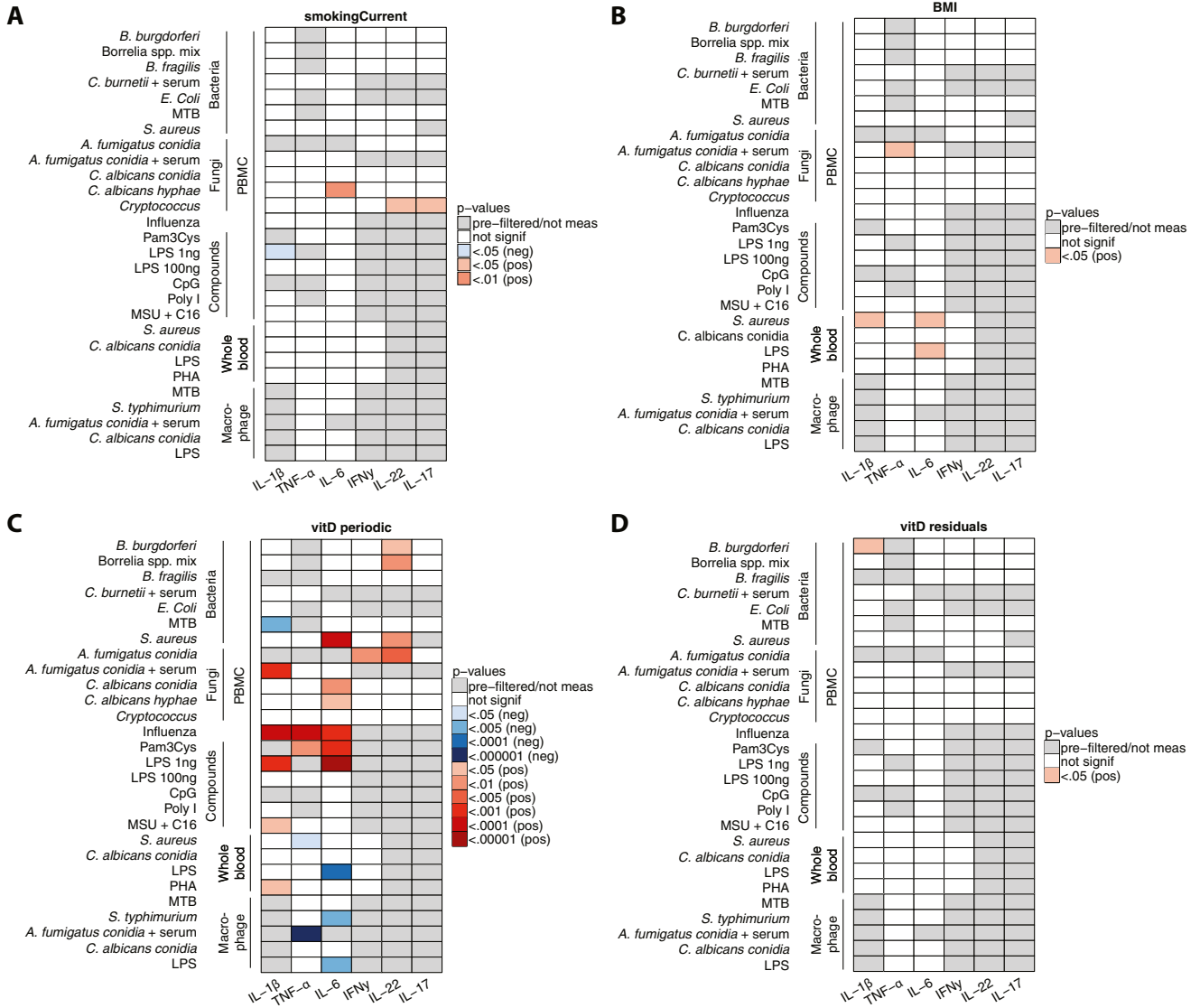
Figure S3. Heatmaps Showing the Relation between Several Factors and Cytokine Production, Related to Figures 4 and 5
(A–D) The factors are: (A) smoking, (B) BMI, (C) periodicity of vitamin D, and (D) non-periodic signal of vitamin D.
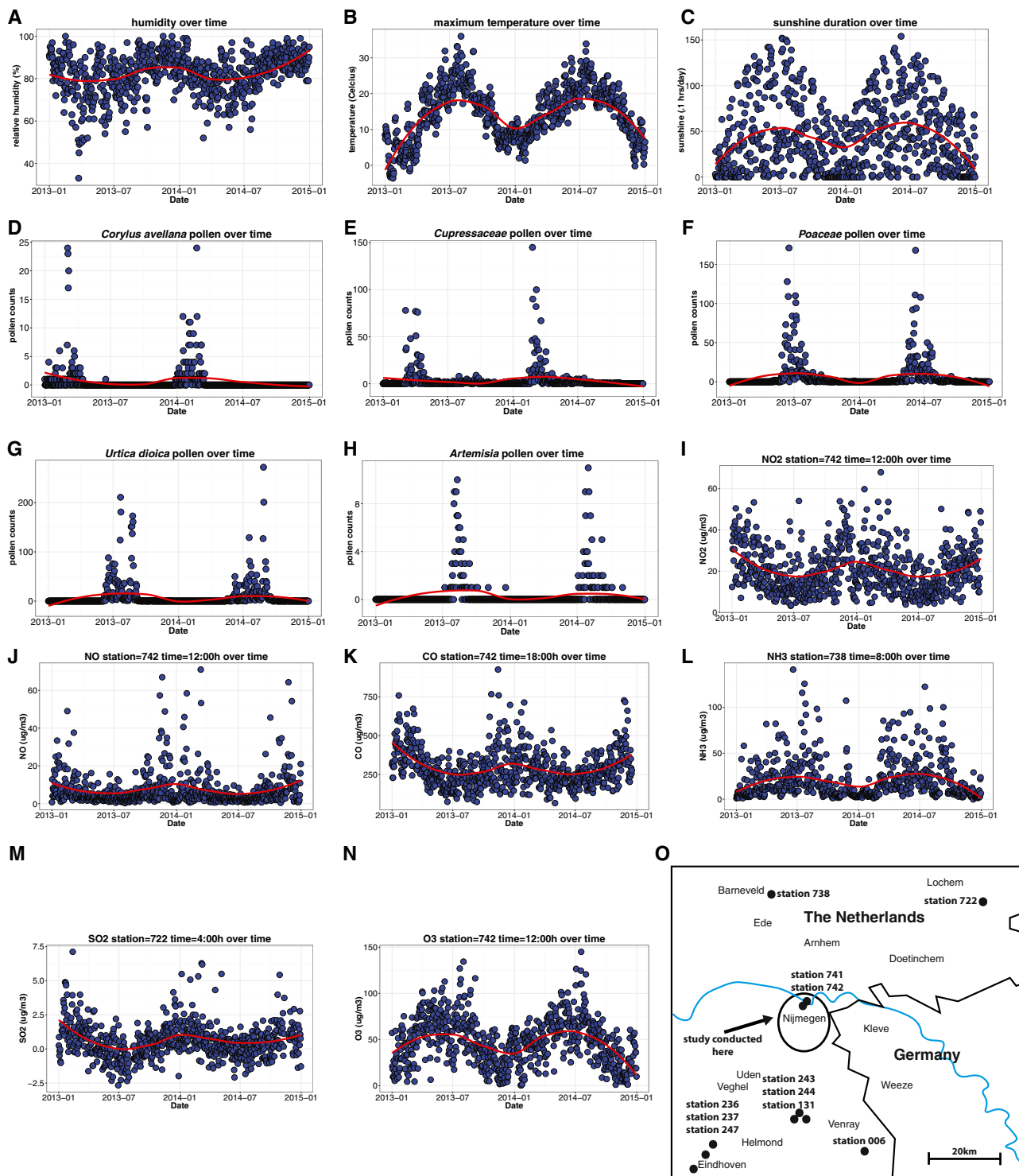
Figure S4. Scatterplots Showing the Seasonality of Different Parameters with a Possible Influence on the Immune System, Related to Figure 5

Red lines indicate the LOESS fit to the data. These data were collected in the time frame our study was conducted.

(A–C) Climatological parameters.

(D–H) Examples of several pollen peaking at different times of year. Pollen were counted as number of pollen from a certain species per m3 of air.

(I–N) Atmospheric concentrations of several compounds in the city where the study was performed.

(O) Location of the measuring stations for the parameters displayed in (I)–(N).
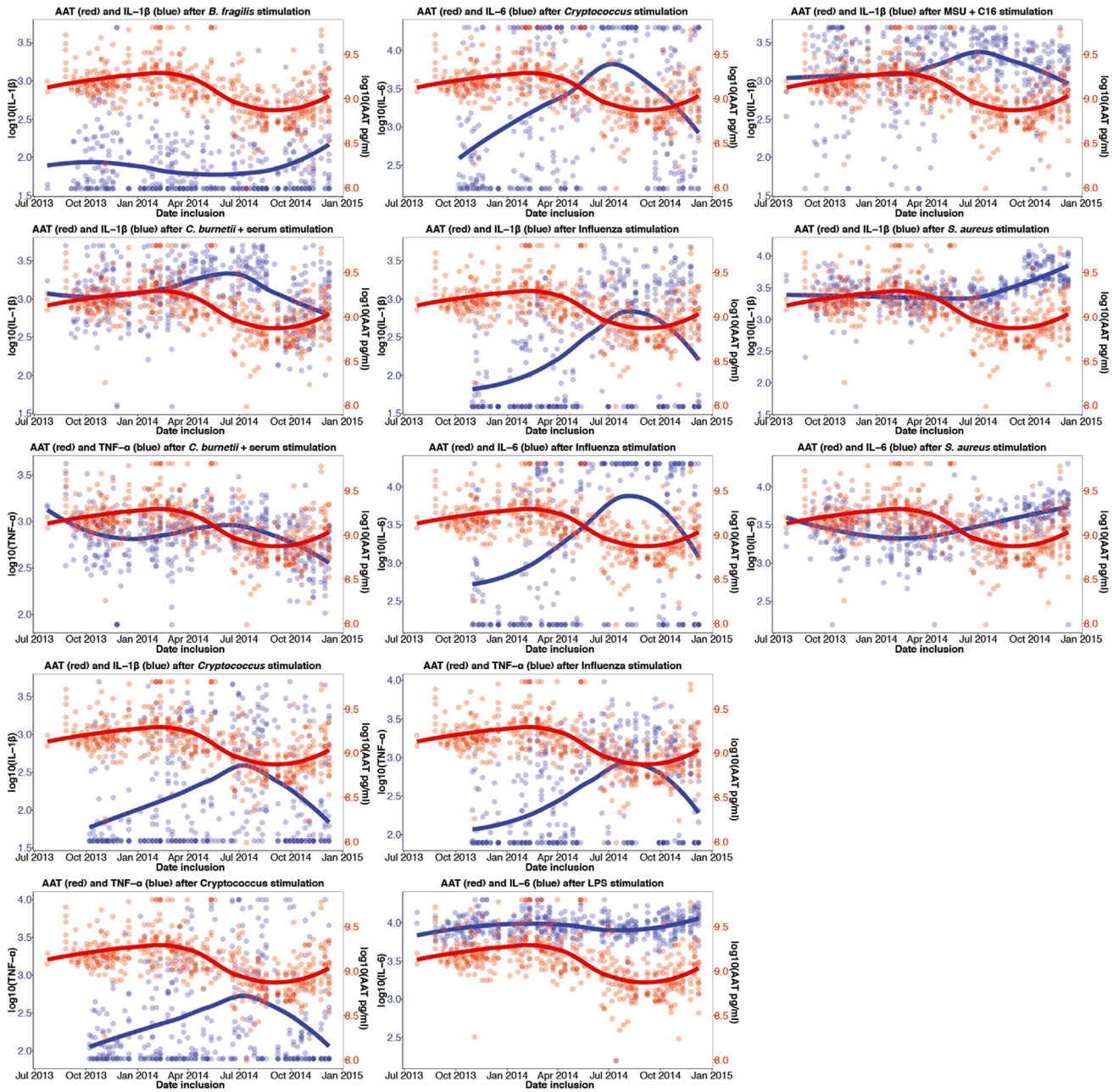
Figure S5. Scatterplots Showing Examples of the Opposed or Similar Seasonality of AAT with Several Cytokine Responses after Stimulation, Related to Figure 6

The lines depict LOESS curves through the scatterplots. Cytokines and stimulations were selected if they showed significant seasonal patterns (see Figure 5) or if they showed clear sample degradation. Examples of significant seasonality include IL-1β after stimulation with *C. burnetii* + serum and IL-6 after Influenza stimulation. Examples of sample storage degradation include IL-1β and IL-6 after *S. aureus* stimulation. All concentrations are in pg/ml.