Cytokine Polymorphisms Have a Synergistic Effect on Severity of the Acute Sickness Response to Infection

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Background. Functional polymorphisms in immune response genes are increasingly recognized as important contributors to the marked individual differences in susceptibility to and outcomes of infectious disease. The acute sickness response is a stereotypical set of illness manifestations mediated by the proinflammatory cytokines induced by many different pathogens. The genetic determinants of severity of the acute sickness response have not previously been explored.

Methods. We examined the impact of functional polymorphisms in cytokine genes with critical roles in the early immune response (tumor necrosis factor–α, interleukin-6, interleukin-10, and interferon-γ) on the severity and duration of illness following acute infection with Epstein-Barr virus, Coxiella burnetii (the causative agent of Q fever), or Ross River virus.

Results. We found that the interferon-γ +874T/A and the interleukin-10 -592C/A polymorphisms significantly affected illness severity, cytokine protein levels, and the duration of illness. These cytokine genotypes acted in synergy to potentiate their influence on disease outcomes.

Conclusions. These findings suggest that genetically determined variations in the intensity of the inflammatory response underpin the severity of the acute sickness response and predict the recovery time across varied infections.

Acute infections are universal and are a leading cause of morbidity, mortality, and economic burden [1]. Despite this, little is known about the reasons why some individuals are resilient to illness, whereas other individuals experience severe acute illness and sometimes a protracted clinical course after acute infection. In most species, the host immune response to acute infection by a wide spectrum of pathogens triggers a stereotypical set of clinical manifestations, including fever, fatigue, hypersomnia, hyperalgesia, anorexia, disturbed mood, and cognitive impairment—termed the acute sickness response [2, 3]. This response combines with manifestations arising from local tissue injury to constitute the symptom manifestations of acute infection.

The universal nature of the acute sickness response documented elsewhere in animals [2], has been confirmed by our group in a cohort of subjects who were observed from the onset of documented acute infection due to Epstein-Barr virus (EBV; the causative agent of infectious mononucleosis), Coxiella burnetii (the causative agent of Q fever), or Ross River virus (RRV; epidemic polyarthritis), until complete recovery. In two separate samples [4, 5], a core set of acute symptoms was evident in all subjects, irrespective of the microorganism responsible for the infection (EBV, a DNA virus that infects B lymphocytes; RRV, a mosquito-borne RNA virus that infects synovial macrophages; and C. burnetii, a zoonotic bacterium that infects tissue macrophages). However, the reported severity of these symptoms and their functional impact varied significantly between individuals, ranging from relatively mild to severe, debilitating illness requiring hospitalization. Illness severity correlated strongly with the levels of IL-1β and IL-6 detected in unstimulated cultures of...
 PBMCs, which extends existing evidence from animal studies to confirm a key role for proinflammatory cytokines as determinants of the well-recognized individual differences in the symptomatic response to infection in humans [4]. In contrast, analysis of the influence of psychosocial and environmental factors on illness outcomes (including demographic characteristics, socioeconomic status, personality, coping style, mood, and psychiatric history) showed no significant effects [5].

Twin and family studies indicate that there is a significant genetic contribution to variations in the host response to immunogenic stimuli [6, 7]. For example, heritability estimates for lipopolysaccharide (LPS)–stimulated production of proinflammatory cytokines from PBMCs were found to be 53%, 57%, and 87% for TNF-α, IL-6, and IL-1β, respectively [7]. Seminal research with the Danish Adoption Register identified a relative risk of 5.8 of an adopted child dying of infection if the biological parent had also died of infection before the age of 50 years—the comparable relative risks for death of cancer or cardiovascular disease were 1.2 and 4.5, respectively [8].

Functional single nucleotide polymorphisms (SNPs) in the proinflammatory cytokine genes, IL-6 (−174G/C) and TNF-α (−308G/A), as well as SNPs that affect the production of anti-inflammatory cytokines, such as IL-10 (−1082G/A, −819C/T, and −592C/A), have been associated with both susceptibility to and outcomes of infectious diseases, including EBV, mycobacterial infections (leprosy and tuberculosis), malaria, leishmaniasis, and bacterial sepsis [9–14]. Polymorphisms in cytokine genes that regulate adaptive immune responses have also been associated with disease response. For example, a SNP in the first intron of the IFN-γ gene (+874T/A) that is known to modulate gene expression [15] has been associated with outcomes of tuberculosis, malaria, leprosy, and severe acute respiratory syndrome, as well as the severity of respiratory syncytial virus infection [9, 16–19].

These data suggest that polymorphisms in cytokine genes altering pro- and anti-inflammatory cytokine production exert a broad influence on the host response to a diverse range of infections, thereby modulating the severity and course of the acute sickness response. In this study, we examined the impact of functional polymorphisms in the TNF-α, IL-6, IL-10 and IFN-γ genes on the severity and duration of illness in patients with documented infection with EBV, RRV, or C. burnetii.

PATIENTS AND METHODS

Participants. Participants included 300 caucasian subjects (mean age, 34 years [range, 16–77 years]; 47% women) from the ongoing Dubbo Infection Outcomes Study [5]. The Dubbo Infection Outcomes Study cohort is composed of subjects with serologically confirmed acute EBV, RRV, or Q fever who are observed from shortly after onset of symptoms until recovery [5]. Written informed consent was obtained from all subjects. The relevant institutional review boards approved the study.

Derivation of the severity phenotype. An index quantifying the severity of the acute sickness response was empirically derived by a principal components analysis of data obtained during acute infection with use of the Somatic and Psychological Health Report [20] and a Physical Symptoms Checklist, which record a wide range of physical and psychological symptoms. This severity index featured the typical dimensions of the acute sickness response, including fever, fatigue, sleep disturbance, pain, loss of appetite, disturbed mood, and impaired concentration. Multiple regression analysis revealed no substantive predictive relationship between the type of infection or demographic factors (i.e., age and sex) and illness severity ($R^2 = 0.004; P = A$). In contrast, strong correlations were demonstrated between illness severity and outcome measures of functional impairment (days in bed, $r = 0.54$; days out of role, $r = 0.35$); $P < 0.001$ for both).

The comparison groups for this study were selected to optimally represent the extremes (the top and bottom one-third) of this severity distribution, as advocated by Sham et al. [21]. The difference in mean symptom scores (±SD) of the resulting high (1.13 ± 0.33) versus low (−1.11 ± 0.40) severity groups was highly significant ($t = 47.5; P < .001$; effect size, 7.5). Because these patients were recruited from the same region and were selected to be ethnically uniform, the risk of spurious findings attributable to population stratification is minimal.

DNA collection and genotyping. DNA was extracted from PBMCs (Wizard DNA kit; Promega) and was quantified using NanoDropR ND-1000 (Biolab), and the quality was verified by agarose gel electrophoresis. DNA samples were genotyped for the TNF-α (−308G/A, IL-6 (−174G/C, IFN-γ +874T/A, and IL-10 (−1082G/A, −819C/T, and −592C/A) SNPs (table 1). Because the IL-10 (−819C/T and −592C/A SNPs are in complete linkage disequilibrium [14, 22], only the SNP at position −592 was included. Genotyping was performed using PCR-based methods (Autoflex; Sequenom) at the Australian Genome Research Facility (Brisbane).

Cytokine quantification. Blood samples were collected and processed under strict endotoxin-minimized conditions. PBMCs were separated (Lymphoprep; AXIS-SHIELD); were cryopreserved in RPMI 1640 media (GIBCO), with 10% dimethyl sulfoxide (Sigma-Aldrich) and 50% autologous plasma; and were stored in vapor-phase nitrogen. After thawing, PBMCs were resuspended in RPMI 1640 medium, supplemented with 10% low-endotoxin, heat-inactivated fetal bovine serum (JRH Biosciences), at 2 × 10^6 PBMCs/mL and were cultured for 24 h at 37°C in medium alone, in LPS (10 ng/mL, from Salmonella typhimurium; Sigma-Aldrich), or in medium with mouse anti-human, anti-CD3, and anti-CD28 Dynabeads (beads:cell ratio, 4:1; Dynal Biotech). Supernatants were stored at −80°C until derivation of the severity phenotype. An index quantifying the severity of the acute sickness response was empirically derived by a principal components analysis of data obtained during acute infection with use of the Somatic and Psychological Health Report [20] and a Physical Symptoms Checklist, which record a wide range of physical and psychological symptoms. This severity index featured the typical dimensions of the acute sickness response, including fever, fatigue, sleep disturbance, pain, loss of appetite, disturbed mood, and impaired concentration. Multiple regression analysis revealed no substantive predictive relationship between the type of infection or demographic factors (i.e., age and sex) and illness severity ($R^2 = 0.004; P = A$). In contrast, strong correlations were demonstrated between illness severity and outcome measures of functional impairment (days in bed, $r = 0.54$; days out of role, $r = 0.35$); $P < 0.001$ for both).

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assayed for the concentrations of the cytokines IL-1β, IL-6, IL-10, TNF-α, and IFN-γ (BioPlex; BioRad).

**Statistical analyses.** Statistical analyses were performed using SPSS for Windows, version 14 (SPSS). Multiple regression analysis was used to confirm independence of the severity index from age, sex, and type of infection. Pearson correlations were sought between illness severity and disability. Associations between genotype and illness severity were analyzed using χ² tests. Protein production by genotype was analyzed using analysis of variance. Time-course analyses (Kaplan-Meier) were used to assess the impacts of illness severity and patient genotype on illness duration.

**RESULTS.** Individuals were excluded from the study in the case of ambiguous ethnicity (31 persons) or incomplete data sets (62 persons). The 300 participants in the study did not differ from the excluded individuals with regard to age (mean age, 35 years [range, 16–77 years]; P = .63) or sex (43% women; P = .43). There was also no difference in severity scores (t = −0.66; P = .51).

**Characteristics of acute infective illness for subjects in severity phenotype extremes.** Table 2 illustrates the substantive differences between individuals with the high versus low severity phenotypes in symptoms of the acute sickness response. There were consistent and statistically significant differences in reported fever, sleep, fatigue, pain, poor appetite, disturbed mood, and impaired concentration, as well as the level of incapacity during the acute illness, which is reflected in the number of days spent in bed or spent missing from work or other role (P < .001 for all variables). A very similar pattern was evident when severity extremes were compared separately for each type of infection.

**Cytokine genotype and severity of acute infective illness.** All genotypes were distributed in Hardy-Weinberg equilibrium (χ² < 1; P > .60). There was a significant association between the high illness-severity phenotype and the IFN-γ +874T allele (OR, 2.5; 95% CI, 1.3–4.7; for the IFN-γ +874TT genotype, OR, 3.0; 95% CI, 1.3–6.5; P = .004). A more modest association was found between the IL-10 −592C/A SNP and the low illness-severity phenotype. Individuals homozygous for the IL-10 −592C allele (IL-10 −592CC genotype) had a lower risk of experiencing severe symptoms during the acute illness than did subjects with either the IL-10 −592CA or −592AA genotypes (OR, 1.9; 95% CI, 1.3–3.3; P = .03). These associations were valid for all 3 subcohorts. None of the other polymorphisms were significantly associated with illness severity.

**IL-10 −592C/A SNP potentiates the effect of IFN-γ genotype on illness severity.** Individuals homozygous for the IFN-γ +874T allele (i.e., IFN-γ +874TT genotype; high IFN-γ production) who also had an A allele at IL-10 −592 (i.e., IL-10 −592TT genotype; high IFN-γ production) had a striking increase in the risk of experiencing a severe illness, compared with individuals who were homozygous for both the IFN-γ +874A (i.e., IFN-γ +874AA genotype) and the IL-10 −592C allele (i.e., IL-10 −592CC genotype) (OR, 8.0; 95% CI, 2.2–28.6; P = .001). The risk of experiencing a more severe acute illness for individuals with this “high risk” IFN-γ +874TT and IL-10 −592 CA or AA genotype was more than double that associated with the IFN-γ +874TT genotype alone.

**Impact of genetic variation on cytokine protein production.** To confirm previous reports of genetically determined differences in IFN-γ and IL-10 production [14, 15, 23], cytokine production was studied in a subset of the study cohort (n = 35). Significantly higher levels of IFN-γ production were found

**Table 1. Sequences of the areas surrounding single nucleotide polymorphisms (SNPs) associated with cytokine genes.**

<table>
<thead>
<tr>
<th>SNP position</th>
<th>SNPα</th>
<th>Sequenceb (5′–3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10 −1082</td>
<td>rs1800896</td>
<td>CACACACACACACAAATCCAAAGACACACTAAGGCTTTGGGA/A/GGGGAAATAGGAGGATAGTAAGGAAAGTAAGGAGCCTCCTA-</td>
</tr>
<tr>
<td>IL-10 −592</td>
<td>rs1800872</td>
<td>AATGAAATCGGGTAAAGAGGCTGGACACATCTTGAGCCCGCTGTG/C/ACGTTAAGACGAGCTTTAGTGTCAGG/G/</td>
</tr>
<tr>
<td>IL-6 −174</td>
<td>rs1800795</td>
<td>TAGCTCTAACAGAACCTAAGCTGACCTTTCCCCTAGTTGTCTTGCG/G/</td>
</tr>
<tr>
<td>IFN-γ +874</td>
<td>rs2430561</td>
<td>ATATTCAGACATTACCAATTAGTTTATTCTACAACACAAATAAATCT/T/ACACACACACACACACACACACACACACTCGACATGTTTTGGAAACTACGGG</td>
</tr>
<tr>
<td>TNF-α −308</td>
<td>rs1800629</td>
<td>ACAGACCTGTCCCAAAAGAAATGGGAGGGAATGTTTGGAGGCGAT/G/AAGGAGGCGTTAGGCTCCTACACACAAATCAGTCA-GTGCCCCA</td>
</tr>
</tbody>
</table>

α National Center for Biotechnology Information SNP database accession number.

β SNPs are shown in bold italics.
Table 2. Clinical features of the acute sickness response for subjects at the illness-severity phenotype extremes.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>All subjects (n = 200)</th>
<th>EBV infection (n = 85)</th>
<th>RRV infection (n = 59)</th>
<th>Q fever (n = 56)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High severity</td>
<td>Low severity</td>
<td>High severity</td>
<td>Low severity</td>
</tr>
<tr>
<td>Fever</td>
<td>58</td>
<td>22</td>
<td>&lt;.001</td>
<td>62</td>
</tr>
<tr>
<td>Hypersomnia</td>
<td>97</td>
<td>48</td>
<td>&lt;.001</td>
<td>96</td>
</tr>
<tr>
<td>Poor sleep</td>
<td>82</td>
<td>31</td>
<td>&lt;.001</td>
<td>84</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>64</td>
<td>29</td>
<td>&lt;.001</td>
<td>75</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>85</td>
<td>21</td>
<td>&lt;.001</td>
<td>71</td>
</tr>
<tr>
<td>Myalgia</td>
<td>92</td>
<td>30</td>
<td>&lt;.001</td>
<td>91</td>
</tr>
<tr>
<td>Headaches</td>
<td>63</td>
<td>12</td>
<td>&lt;.001</td>
<td>64</td>
</tr>
<tr>
<td>Disturbed mood</td>
<td>97</td>
<td>48</td>
<td>&lt;.001</td>
<td>96</td>
</tr>
<tr>
<td>Impaired concentration</td>
<td>79</td>
<td>22</td>
<td>&lt;.001</td>
<td>80</td>
</tr>
<tr>
<td>Fatigue after activity</td>
<td>100</td>
<td>47</td>
<td>&lt;.001</td>
<td>100</td>
</tr>
<tr>
<td>Fatigue despite rest</td>
<td>97</td>
<td>23</td>
<td>&lt;.001</td>
<td>96</td>
</tr>
</tbody>
</table>

Mean duration in bed, days (95% CI): 
- All subjects: 8.1 (6.4–9.7) days, <.001
- EBV infection: 9.5 (6.9–12.2) days, <.001
- RRV infection: 5.5 (3.8–7.2) days, .01
- Q fever: 5.0 (2.4–7.3) days, .002

Mean duration out of role, days (95% CI): 
- All subjects: 18.4 (16.6–20.3) days, <.001
- EBV infection: 20.2 (17.4–23.1) days, <.001
- RRV infection: 9.4 (7.3–11.4) days, <.001
- Q fever: 16.2 (12.6–19.8) days, <.001

**NOTE.** Data are percentage of patients, unless otherwise indicated. Symptoms are presented as the percentage of subjects who reported being “troubled over the past few weeks” by the symptom “a good part of the time” or “most of the time” on the Somatic and Psychological Health Report questionnaire [20]. EBV, Epstein-Barr virus; RRV, Ross River virus.

* Percentage of individuals who experienced mood disorder was determined by the PSYCH subscale of the Somatic and Psychological Health Report questionnaire [20].

* Functional impairment as reported in the Brief Disability Questionnaire.
in stimulated cultures of PBMCs from persons homozygous for the IFN-γ +874T allele (high illness severity), compared with PBMCs from persons with an IFN-γ +874 AA or AT genotype (with anti-CD3/CD28, P = .009; with LPS, P = .001; figure 1A and 1B). Stimulated cell cultures from persons homozygous for the IFN-γ +874T allele also contained higher levels of the proinflammatory cytokines IL-1β and TNF-α, compared with cultures from persons with an IFN-γ +874 AA or AT genotype (with LPS, P = .03 for IL-1β and P = .035 for TNF-α; figure 2A and 2B). There were no significant differences in cytokine production in unstimulated PBMC cultures.

With regard to the IL-10 genotype, in individuals homozygous for the IL-10 −592C allele, there was a trend for higher levels of IL-10 in unstimulated PBMC cultures, and there were significantly lower levels of the proinflammatory cytokines IL-6 (P = .007) and TNF-α (P = .01) in LPS-stimulated cultures (i.e., IL-10 −592CC genotype; low illness severity), compared with levels detected in cultures from persons with an IL-10 −592 AA or CA genotype (figure 2C and 2D). There were no significant differences in IL-10 production detected in PBMCs stimulated with anti-CD3/CD28.

The IFN-γ +874TT genotype (high IFN-γ activity) combined with any A at position −592 of IL-10 (IL-10 −592 AA or CA genotype; low IL-10 activity) was also associated with significantly higher levels of IFN-γ (with LPS, P = .01) and TNF-α (P = .04), compared with the “low risk” genotype combination of IFN-γ +874AA and IL-10 −592CC (figure 1C).

**Illness severity predicts illness duration.** Time-to-recovery analyses revealed a powerful relationship between the illness-severity phenotype and the total duration of symptomatic illness (χ² = 124, P < .001). Individuals with the low illness-severity phenotype experienced symptoms for a median of 20 days, whereas those with the high illness-severity phenotype were symptomatic for 135 days. The combination of IFN-γ and IL-10 genotypes also impacted the duration of illness. Persons with the “low risk” genotype (IFN-γ +874AA and IL-10 −592CC) were symptomatic for 34 days, but persons with the “high risk” genotype (IFN-γ +874TT and IL-10 −592 AA or CA) experienced symptoms of illness for 80 days (χ² = 5.7; P = .01; figure 3).

**DISCUSSION**

Functional polymorphisms in the IFN-γ and IL-10 loci significantly influence cytokine production, as well as the severity and duration of illness after infection with EBV, RRV, or C.

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**Figure 1.** Boxplots showing production of IFN-γ in PBMC cultures stimulated with anti-CD3/CD28-coated beads (A) or LPS (B and C). Data are presented by IFN-γ +874 genotype in panels A and B; in panel C, data are presented by the genotype combination of IFN-γ +874TT and IL-10 −592AA or IL-10 −592AC (“high risk”) versus the homozygous genotype combination of IFN-γ +874AA and IL-10 −592CC (“low risk”).

**Figure 2.** Boxplots showing production of proinflammatory cytokines by PBMC cultures stimulated with lipopolysaccharide for IFN-γ +874 genotypes (A and B) and IL-10 −592 genotypes (C and D).
These findings strengthen the evidence of a pivotal role of a balanced cytokine response in dealing with acute infections and of strong genetic regulatory effects on that response. Moreover, we have documented that risk-associated cytokine genotypes act in synergy to potentiate their impact on illness severity. Because of the highly varied characteristics of the pathogens studied here, these findings may plausibly be generalized to the host response to many infectious agents.

It should be noted that, in acute infections, complications arising from local tissue injury (relating either to direct effects of the pathogen or to immunopathology) may also give rise to illness manifestations. In some cases such manifestations may dominate the illness complex (e.g., microcirculatory changes leading to coma in cerebral malaria), although these are typically accompanied by the systemic features of the acute sickness response, such as fever; it is the latter manifestations that are stereotypical across different infections and are the focus of this analysis. Furthermore, in many cases, both the local and systemic manifestations are mediated by the same pro- and anti-inflammatory cytokines.

These findings are concordant with the substantial evidence for the central role of both IFN-γ and IL-10 in determining the characteristics of the innate and adaptive host responses to a wide range of pathogens [24, 25] and, therefore, determining susceptibility to many intracellular bacterial, parasitic, and viral infections [26–28]. In relation to the acute sickness response, the potent inhibitory effects of IL-10 on IL-1β and TNF-α production by activated macrophages [29, 30] are noteworthy, because these cytokines often have synergistic activities in the induction of inflammation [25]. In addition, administration of IL-10 in vivo has been shown to inhibit proinflammatory cytokine production [31].

The T allele of the IFN-γ +874T/A SNP not only demonstrated a robust association with the severity of the acute sickness response, but the risk of experiencing a severe acute illness also increased substantially for homozygous carriers of the T allele, compared with heterozygous carriers, suggesting a gene-dosage effect. This is consistent with the observation that the administration of IFN-γ to humans leads to symptoms reminiscent of the acute sickness response [32]. Associations between the high-activity IFN-γ +874TT genotype and illness severity in patients with tuberculosis [16] and respiratory syncytial virus infection [19] have been reported elsewhere. Furthermore, increased production of both IFN-γ and proinflammatory cytokines has been associated with severe infectious mononucleosis [33] and tuberculosis [34]. IFN-γ and TNF-α have also been implicated in driving the immunopathological process leading to life-threatening cerebral complications associated with severe malaria, whereas IL-10 appeared to work against this process [35].

This present study revealed an association between the low IL-10-production genotype and the SNP at nucleotide position −592 but not the SNP at −1082. Previous studies that examined the impact on infectious diseases outcomes of the 3 prevalent SNPs (at nucleotide positions −1082, −819, and −592) in the IL-10 promoter produced inconsistent results that were potentially attributable to confounding immunogenetic influences [13]. For example, Helminen et al. [12] identified the low-production IL-10 −1082G allele in 26 (72%) of 36 individuals who experienced severe infectious mononucleosis; this allele was identified in only 28 (54%) of 52 asymptomatic EBV-seropositive adults. However, no details were provided as to whether the EBV-seropositive control subjects had previously had severe infectious mononucleosis, and their age (a recognized correlate of the severity of acute infectious mononucleosis) was not noted [36]. In contrast, a highly significant association was found between survival in intensive care units and the IL-10 −592C/A SNP but not the IL-10 −1082G/A SNP. In addition, a strong association was documented between the IL-10 −592A allele, significantly lower IL-10 release, and increased mortality in these patients [14].

Although the contribution of the IL-10 −592C/A SNP to illness severity was modest, individuals with any A allele at −592 and the IFN-γ −874TT genotype had 8-fold greater odds of a severe illness response. This discovery of a significant risk potentiation for the “high risk” genotype combinations points to the importance in the field of immunogenetics of moving beyond the study of individual cytokines to examination of the complex microenvironment resulting from the interaction of several genes and gene products.

Examination of cytokine protein production confirmed the functional significance of the reported genetic associations. In addition to expected changes in the levels of IFN-γ and IL-10,
cell cultures of individuals with genotypes that would favor more intense inflammatory responses (high IFN-γ, low IL-10 production) also demonstrated higher levels of proinflammatory cytokines, including IL-1β, TNF-α, and IL-6, which suggests a feedback mechanism of IFN-γ (produced by T lymphocytes) affecting production of these proinflammatory cytokines (likely predominantly produced by monocytes).

This premise is consistent with our findings of a more severe acute illness and delayed recovery associated with the IFN-γ +874T allele. These outcomes diverge somewhat from those of murine studies in the lymphocytic choriomeningitis virus model [37] in which experimental reduction of IL-10 activity was found to facilitate sustained T cell responses (including IFN-γ production) and more-rapid clearance of the microorganism and, therefore, a shorter course of illness. By comparison, in the data set described here, the high IFN-γ and low IL-10 production genotype was associated with a more severe acute phase and a longer duration of illness. However, this is in keeping with other evidence from the Dubbo Infection Outcomes Study cohort, in which prolonged illness was not found to be associated with either ongoing viral activity or ongoing cytokine production in the peripheral blood [38, 39].

The acute sickness response is initiated by production of proinflammatory cytokines but is ultimately mediated by neurochemical changes in the brain [3]. The mechanisms by which peripheral cytokine signals are transmitted to the brain are not completely understood but include direct neural pathways (via primary autonomic afferents) and cytokine entry at brain regions where the blood-brain barrier is incomplete [40–42]. Animal studies indicate that peripheral cytokines induce de novo synthesis of cytokines from resident microglia and astrocytes [43, 44]. The mechanisms for translation of these cytokine signals into altered neural transmissions remain unclear. Of interest to the association we observed between severity of the acute sickness response and the duration of illness manifestations is the growing awareness of a prevalent microglial response to a range of infectious and inflammatory stimuli via Toll-like receptors (reviewed in [45]) and the recognition that a single exposure to LPS-induced TNF-α may trigger microglial activation that is sustained for 10 months in the absence of systemic inflammation [46]. Accordingly, we suspect that in individuals with a prolonged postinfective illness, a prominent cytokine response in the acute phase causes sensitization in these CNS pathways, which leads to sustained neurobehavioral phenomena that manifest with ongoing symptoms in the absence of persistent peripheral cytokine production.

The challenge faced by the immune system of an infected host is to respond with sufficient intensity and duration to control or eliminate the pathogen, while minimizing both short-term and sustained immunopathological injuries. Our results demonstrate that common polymorphisms in host response genes have an impact on the acute sickness response to infection. Those individuals whose genetic makeup favors a more intense inflammatory reaction are likely to experience more severe symptoms of infectious disease and a more protracted illness. Further studies to elucidate additional immunological and neurobehavioral genes associated with the acute sickness response are warranted. Ultimately, discovery of combination haplotypes associated with the acute sickness response and investigation of the related biological processes may allow identification of individuals at high risk of severe and prolonged illness after infection to inform individualized prevention and treatment approaches for common infectious diseases.

DUBBO INFECTION OUTCOMES STUDY GROUP

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