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Influence of Polymorphism in the Genes for the Interleukin (IL)-1 Receptor Antagonist and IL-1β on Tuberculosis

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Summary
Several lines of evidence suggest that host genetic factors controlling the immune response influence infection by Mycobacterium tuberculosis. The proinflammatory cytokine interleukin (IL)-1β and its antagonist, IL-1Ra (IL-1 receptor agonist), are strongly induced by M. tuberculosis and are encoded by polymorphic genes. The induction of both IL-1Ra mRNA and secreted protein by M. tuberculosis in IL-1Ra allele A2-2-positive (IL-1Ra A2+ ) healthy subjects was 1.9-fold higher than in IL-1Ra A2-2 subjects. The M. tuberculosis-induced expression of mRNA for IL-1β was higher in subjects of the IL-1b (+3953) A1+ haplotype (P = 0.04). The molar ratio of IL-1Ra/IL-1β induced by M. tuberculosis was markedly higher in IL-1Ra A2+ individuals (P < 0.05), with minor overlap between the groups, reflecting linkage between the IL-1Ra A2 and IL-1b (+3953) A2 alleles. In M. tuberculosis-stimulated peripheral blood mononuclear cells, the addition of IL-4 increased IL-1Ra secretion, whereas interferon γ increased and IL-10 decreased IL-1β production, indicative of a differential influence on the IL-1Ra/IL-1β ratio by cytokines. In a study of 114 healthy purified protein derivative-reactive subjects and 89 patients with tuberculosis, the frequency of allelic variants at two positions (−511 and +3953) in the IL-1b and IL-1Ra genes did not differ between the groups. However, the proinflammatory IL-1R α A2−/IL-1b (+3953) A1+ haplotype was unevenly distributed, being more common in patients with tuberculous pleurisy (92%) in comparison with healthy M. tuberculosis-sensitized control subjects or patients with other disease forms (57%, P = 0.028 and 56%, P = 0.024, respectively). Furthermore, the IL-1R α A2− haplotype was associated with a reduced Mantoux response to purified protein derivative of M. tuberculosis: 60% of tuberculin-negative patients were of this type. Thus, the polymorphism at the IL-1 locus influences the cytokine response and may be a determinant of delayed-type hypersensitivity and disease expression in human tuberculosis.

Key words: interleukin 1 receptor • tuberculosis • susceptibility, disease • hypersensitivity, delayed • granuloma

By comparison with other pathogens, widely distributed isolates of M. tuberculosis show a striking lack of antigenic variation (1). The occurrence of tuberculosis epidemics in populations previously unexposed to M. tuberculosis (2, 3) and the twofold risk of disease in identical, compared with nonidentical, twins (4) indicates a genetic component in susceptibility. Rare susceptibility to recurrent atypical intracellular infection is proved to be conferred by mutation in the genes for the IFN-γ receptor (5-7), the IL-12 receptor (8, 9), or IL-12 (10). However, the extent to which these severe defects contribute to susceptibility in populations is unknown. In a recent large case-control study, disease susceptibility in West Africans was conferred by variants of the human Namp1 and vitamin D receptor genes (11, 12). The possibility also exists that disease expression, as well as susceptibility to tuberculosis per se, is influenced by the host response. A single genetic isolate of M. tuberculosis associated with a disease outbreak caused highly varied disease manifestations (13), and in earlier population-based studies, severe pulmonary tuberculosis has been associated with both HLA-DR15 and haptoglobin 2-2 (for review, see reference 14).

A key element in the inflammatory response is the prompt production of proinflammatory cytokines such as IL-1β and TNF-α, required to control infection by M. tuberculosis (15, 16). To terminate the immune response and limit the potential for immunopathology, the proinflammatory response is in turn downregulated by cytokines such as...
TGF-β, IL-10, and, specifically in the case of IL-1β, the IL-1 receptor antagonist (IL-1Ra), a pure antagonist of the IL-1 type 1 receptor (IL-1R1) (17). The genes coding for both IL-1β and the IL-1Ra gene are on chromosome 2q. Two allelic polymorphisms in the IL-1β gene at positions −511 and +3953 relative to the transcriptional start codon have been described (18, 19). Allele 1 of the +3953 polymorphism (IL-1b +3953 A1−) is associated with moderately increased IL-1β production in response to LPS (19). The IL-1Ra gene is also polymorphic due to a variable number (2–6) of tandem repeats of 86 bp (VNTR) within its second intron (20). This polymorphism has been shown to be unambiguously functional at the level of secreted protein, as monocytes from individuals homo- or heterozygous for allele 2 (IL-1Ra A2−, IL-1RN N2, 2 repeats) produce significantly more IL-1Ra in response to GM-CSF (21) and also have higher plasma levels (22). Serum IL-1Ra is known to be elevated in patients with response to GM-CSF (21) and also have higher plasma levels (22). Serum IL-1Ra is known to be elevated in patients with tuberculosis (23). In addition, the ratio of IL-1Ra to IL-1β is elevated in the cerebrospinal fluid of cases of tuberculosis, as compared with pyogenic meningitis (24). These data suggest that the expression of IL-1Ra may impact on disease expression. However, the effect of M. tuberculosis infection on the secretion of IL-1β and IL-1Ra in vitro has not been related to these polymorphisms nor has the relevance of the latter to tuberculosis been investigated.

In this study, we found that M. tuberculosis-induced IL-1Ra mRNA and protein secretion in healthy IL-1Ra A2− subjects was approximately twofold that of IL-1Ra A2+ individuals. In addition, the molar ratio of IL-1Ra a/IL-1β was strikingly higher in IL-1Ra A2+ individuals. In M. tuberculosis-stimulated PBMCs, the addition of IL-4 increased IL-1Ra secretion, whereas IFN-γ increased, and IL-10 decreased, IL-1β production, indicative of a differential influence on the IL-1Ra a/IL-1β ratio by cytokines. In a pilot case-control analysis, the IL-1β and IL-1Ra allele frequencies were not different between patients with tuberculosis and purified protein derivative (PPD) skin test (M antoux)-reactive control subjects. However, the proinflammatory IL-1Ra A2−/IL-1β (+3953) A1− haplotype was unevenly distributed, being more common in patients with pleural tuberculosis and less common in extrapulmonary disease. Furthermore, and consistent with the in vitro observations, the IL-1Ra A2− haplotype was associated with a reduced M antoux response: 60% of tuberculin-nonreactive patients were of this type. Thus, the polymorphism at the IL-1 locus influences the cytokine response to, and may be a determinant of, delayed-type hypersensitivity (DTH) and disease expression in human tuberculosis.

Materials and Methods

Study Populations. For cell culture, healthy, PPD skin test-negative donors from the laboratory staff at Case Western Reserve University were bled and genotyped as below. In the pilot case-control analysis, a different population of 89 unselected pa-

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1A abbreviations used in this paper: DTH, delayed-type hypersensitivity; PPD, purified protein derivative; rt, room temperature.
C t cell culture. PBMCs were separated over a Ficoll (Phar-
macia Biotech) gradient. Preliminary experiments established that
conventional separation of monocytes by adherence to plastic,
harvesting, and replating led to spontaneous release of IL-1Ra. To
reduce such activation, freshly isolated PBMCs were cultured
harvesting, and replating led to spontaneous release of IL-1Ra.
conventional separation of monocytes by adherence to plastic,
samples and dilutions of standard cytokines were then incubated
appropriate isotype control antibodies were purchased from R & D
lysates was consistently
ratio of IL-1Ra secreted into the supernate to that remaining in cell
then freeze-thawing once. In this way we established that the ra-
cells had been removed by washing and wells containing unsepa-
ratied PBMC, indicating that the adherent cells were responsible
for the IL-1Ra secretion. We therefore collected, and froze at
−70°C, PBMC supernates after 10 h of culture. In some cases,
cell lysates were prepared by adding an equal volume of PBS and
then freeze-thawing once. In this way we established that the ra-
of IL-1Ra a secreted into the supernate to that remaining in cell
lysates was consistently >10:1, irrespective of time point, stimu-
lus, and genotype.
R egents. M. tuberculosis H37R a and H37R v was prepared and
 aliquoted as previously described (26). Aliquots were vor-
texted for 15–20 min before use at an infection ratio of 0.1 or 1
M. tuberculosis bacilli/1 PBMC (corresponding to ~1:1 and 10:1
per monocyte). PPD of M. tuberculosis was the gift of Lederle
Labs. (American Cyanamid Co.) and used at 0.1–100 μg/ml.
R recombinant TGF-β, IL-4, and IFN-γ, and the neutralizing an-
tibodies to IL-1β (mouse IgG1), IL-6 (polyclonal goat IgG)
TGF-β (polyclonal chicken IgY), and TNF-α (mouse IgG1), and
appropriate isotype control antibodies were purchased from R & D
Systems, Inc. All recombinant cytokines, PPD, M. tuberculosis,
and neutralizing antibodies used were tested for endotoxin con-
tamination by the Limulus amebocyte assay (Biowhittaker) and
were either free or contained very small levels (always <2 ng/mg)
of endotoxin.
Cytokine ELISA. Maxisorp (Nunc, Inc.,) plates were coated
overnight at 4°C with 100 μl of the following coating antibodies
in PBS: 2 μg/ml anti-human IL-1β mAb or 5 μg/ml of anti-
human IL-1Ra mAb (both from R & D Systems, Inc.). After
washing in PBS/0.05% Tween 20 (×3), the plates were blocked for
1 h at room temperature (rt) using 300 μl 1% BSA/5% sucrose/
0.05% NaN3 in PBS. After three further washes, duplicate 100-μl
samples and dilutions of standard cytokines were then incubated
for 2 h at rt. After washing (×4), 100 μl of the following biotiny-
lated detection antibodies were added in diluent (0.1% BSA,
0.05% Tween 20 in TBS, pH 7.3): 100 ng/ml anti-human IL-1β
antibody or 20 ng/ml anti-human IL-1α antibody (both from
R & D Systems, Inc.). After 2 h at rt, the plates were washed (×5)
and 100 μl streptavidin horseradish peroxidase (Jackson Immu-
no research) at 1:5000 in diluent was added. After 20 min, six
final washes were followed by the addition of 100 μl of 3,3′,5,5′-tetra-
methylbenzidine hydrochloride solution in perborate (Sigma
Chemical Co.) to each well. The reaction was stopped by adding
50 μl/well 0.5 N H2SO4, and the plates were read at 450 nm in
an ELISA reader. The sensitivity of each cytokine ELISA was as
follows: IL-1β, <1 pg/ml and IL-1Ra a, 0.05 ng/ml.
R ibonuclease Protection Assay. 5 × 103 freshly isolated PBMCs
were used to obtain ~5 × 106 adherent cells. T his population of
cells is up to 90% monocytes by cytostaining and is 99% viable
(27). After resting overnight, the adherent cells were infected as
above with M. tuberculosis at 1:1. After 4 h, the cells were har-
vested, and total RNA was extracted using guanidinium isothio-
cyanate, CsCl2 density gradient centrifugation, and ethanol pre-
cipitation. 2 μg of the resultant RNA was hybridized overnight
according to the manufacturer’s instructions to a cocktail of
[32P]UTP (Du Pont)-labeled complimentary RNA probes (Phar-
m ingen) for IL-1α, IL-1β, IL-1Ra a, IL-6, IL-10, IL-12 p40 and
p35, TNF-α and -β, TGF-β1-3, LT-β, and the housekeeping
genes L32 and GAPDH at 56°C. Single-stranded RNA was di-
gested by incubation with RNase for 45 min at 37°C and the
protected fragments reextracted by ethanol precipitation. T he
products were electrophoresed on a 5% denaturing polyacrlyl-
amic gel; a negative control RNA and the unhybridized radio-
active probe were run in each experiment. T he gel was exposed
overnight using a Biorad Geldoc 1000. T he identity of the pro-
tected bands was confirmed by reference to the unhybridized
probes and quantitated by reference to bands for the housekeep-
genes L32 and GAPDH.
C F U A ssay for the Intracellular G rowth of M. tuberculosis. T his
assay was performed as previously described with minor modifi-
cations (28). In brief, adherent cells were plated in triplicate wells
in 96-U microtiter plates (Corning Glass Works) and reseeded
for 2 h. Cells were infected with M. tuberculosis H37R a at 1:1, 10:1,
and 100:1 (bacillus/cell) in 30% autologous serum. After 2 h,
noningested bacteria were removed by washing gently (×3) with
prewarmed RPMI 1640. Each well then received RPMI 1640
containing 2% autologous serum, and the plates were cultured in
a humidified incubator at 37°C in the presence of 5% CO2 for
as little as 1 hr (time zero sample) up to 10 d. D uplicate wells con-
tained 2 μg/ml of neutralizing anti-IL-1Ra a (goat IgG; R & D Sys-
tems, Inc.) or the same amount of isotype control antibody. At the
end of the culture period, supernates were aspirated, and the plates
containing the infected adherent cells were frozen at −70°C. T o
determine the number of intracellular bacteria in the CFU assay,
the plates were thawed and cells lysed with 0.25% SDS in PBS
for 12 min and then neutralized using 20% BSA. T he lysates
were then 10-fold serially diluted with 7H 9 broth (Difco Labs., Inc.),
and three 10-μl aliquots of each dilution were plated on Middle-
brook 7H 10 agar (Difco Labs., Inc.). T he plates were then
incubated for 19 d at 37°C in humidified air with 5% CO2. T o
prove the culture period, the number of CFUs in each of the
three replicate spots was enumerated for at least two consecutive
dilutions using a stereomicroscope and averaged. U sing this tech-
nique, extracellular growth of mycobacteria as assessed by culture
of the supernates is consistently >1 log lower than intracellular
growth (26). T he rate of intracellular growth expressed as dou-
bling time was determined by reference to the logarithmic growth
from the cultures.
R esults
Polymorphism in the IL-1Ra a G ene A ssociates with the Stimu-
lated Production of IL-1Ra a. F irst, we examined the M. tuber-
culosis-stimulated production of IL-1Ra a by culture of 2.5 ×

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lated production of IL-1Ra was slightly, but not significantly, higher in the IL-1Ra A2 group (Fig. 1 A). Stimulation by M. tuberculosis (0.1 and 1:1 bacillus/cell) caused a dose-dependent increase in IL-1Ra secretion irrespective of genotype. However, the median response of the IL-1Ra A2 group was 1.9 times greater at both doses of M. tuberculosis tested (P = 0.02 at 1:1). In a subset of 16 healthy subjects, the dose response of IL-1Ra induction to PPD was also determined (Fig. 1 B). Although IL-1Ra A2 individuals showed a dose-dependent increase in IL-1Ra secretion, this did not become statistically significant until the dose of PPD was 100 μg/ml. The response of IL-1Ra A2 individuals was 2.1–3.6 times higher, depending on the dose. In contrast, induction of IL-1Ra in IL-1Ra A2+ donors was significant at 1 μg/ml. Thus, IL-1Ra A2+ donors appeared more sensitive to PPD stimulation. The median production of IL-1Ra in response to PPD (10 μg/ml) was also 1.82 times greater in the IL-1Ra A2+ donors (6.6 ± 1.3 vs. 3.6 ± 0.5 ng/ml/10^6 monocytes, P = 0.012).

Relationship between Polymorphisms and the Production of IL-1β. We next determined the level of IL-1β in the same culture supernates used for the analysis of IL-1Ra. In contrast to the IL-1Ra polymorphism, the two polymorphisms in the IL-1β gene did not correlate with the M. tuberculosis-stimulated production of IL-1β to the same extent. The median M. tuberculosis (1:1)-stimulated production of IL-1β in subjects positive for the −511 A2 (n = 20) was 635 ± 119 pg/ml and 404 ± 261 pg/ml in A1/A1 homozygotes (n = 8). The corresponding figures for the +3953 polymorphism were 404 ± 84 pg/ml (A2+, n = 12) and 643 ± 171 pg/ml (A1/A1 homozygotes, n = 16). IL-1β production did tend to be higher in IL-1Ra A2− subjects, but only significantly so in response to M. tuberculosis at 0.1 μg/ml (P = 0.01) (data not shown).

As a pure antagonist of IL-1, IL-1Ra competes for occupancy of IL-1Rα, and it has been estimated that IL-1Ra needs to be present in a large molar excess (25–50 ×) to antagonize IL-1β significantly (28). Therefore, the ratio of IL-1Rα/IL-1β is likely to be more relevant to regulation of the inflammatory response than the absolute value of either cytokine. The molar ratio of IL-1Rα/IL-1β was therefore calculated for each supernate and was significantly higher in IL-1Ra A2+ individuals (P = 0.05) in response to doses of both M. tuberculosis and PPD at 1, 10, and 100 μg/ml (Fig. 2 B), in some cases with only minor overlap between the groups. By contrast, the response to LPS did not differ significantly between the groups. Fig. 2 B shows that the highest ratios likely to result in antagonism of the IL-1β response to PPD and M. tuberculosis stimulation (especially at lower doses likely to be relevant to M. tuberculosis-infected foci) were observed in the majority of IL-1Ra A2+ individuals but only in a minority of IL-1Ra A2− subjects. The bulk of the experiments were performed using attenuated M. tuberculosis H37Rv in three donors (one A1/A1 and two A1/A2) was also performed. The level of each cytokine was very similar, such that at an infection multiplicity of 1:1 the IL-1Rα/IL-1β ratio when stimulated by H37Rv was 4.1, 16.8, and 12.6 for the three donors and 6.2, 17.3, and 8.0, respectively when stimulated by H37Rv. We thus have no reason to suspect that the findings using M. tuberculosis H37Rv would not apply to virulent clinical isolates.

![Figure 1](https://example.com/figure1.jpg)

**Figure 1.** Association between IL-1Ra genotype and the monocyte production of IL-1Ra in response to M. tuberculosis and PPD. The amount of IL-1Ra produced by PBMCs from healthy, PPD-nonreactive donors during a 10-h co-culture with either M. tuberculosis (A) or PPD (B) was determined by ELISA. Values were normalized to the number of monocytes present in the culture. The response of IL-1Ra A2+ individuals was higher at all doses of either stimuli.
Cytokine Gene Expression by Ribonuclease Protection Assay. We next sought to investigate association between the polymorphisms and the expression of mRNAs. Ribonuclease protection assay was performed on RNA from 13 donors, all of different genotypes. The spontaneous expression of IL-1α and IL-1β transcript was low. There was no constitutive expression of any other monocyte cytokine, indicating that this low expression was unlikely to have been due to a nonspecific effect of cellular activation during isolation. Within 1 h, M. tuberculosis induced IL-1α a gene expression in all individuals irrespective of genotype, together with the mRNAs for IL-1β and TNF-α and followed slightly later (2 h) by IL-1α and IL-6. At hour 4, there was higher induction of IL-1α in the IL-1α A2 subjects consistent with the protein data, although the difference was not statistically significant (Table I). The IL-1β +3953 allele A2 was associated with significantly lower production of IL-1β transcript (P = 0.04). Taken together, we interpret these observations to indicate that the alleles are associated with differences in transcription, but the dissociation between induction and secretion, particularly in the case of IL-1β, indicates that posttranscriptional mechanisms also influence cytokine secretion.

Effect of Monocyte Cytokines on the Production of IL-1α in Response to M. tuberculosis. The results so far showed that in response to M. tuberculosis or its PPD, IL-1α a gene expression is induced within 1 h, large quantities of protein are secreted within 10 h, and differences between individuals could be related to their genotypes. However, an indirect modulating influence of M. tuberculosis via increased translation of preexisting IL-1α mRNA or an effect of other cytokines such as TGF-β, TNF-α, IL-1β, and IL-6 produced by monocytes early in response to infection is also possible. We investigated this possibility by assessing the ability of antibodies known to neutralize the biological effects of TGF-β, TNF-α, IL-1β, and IL-6 on M. tuberculosis-stimulated production of IL-1α. Control wells received isotype-matched antibodies. No consistent effect on constitutive or stimulated IL-1α a secretion was seen, irrespective of genotype, cytokine, or dose of antibody used (up to 1,000-fold the ED₅₀ concentrations). TGF-β modulates the human response to tuberculosis (29, 30) and has also been reported to increase IL-1α in some (31) but not all studies (32). We therefore also evaluated the effect of TGF-β (0.1–10 ng/ml) on both M. tuberculosis-stimulated and -unstimulated IL-1α a production in 12 individuals (6 IL-1α A2 and 6 IL-1α A2 +). No significant enhancement of the early secretion of IL-1α a was seen (data not shown). However, rIL-10 (0.1–10 ng/ml) caused a significant dose-dependent increase in the M. tuberculosis-stimulated IL-1α a/IL-1β ratio in IL-1α A2 + and IL-1α A2 + donors at all doses tested (P < 0.02), an effect largely due to the suppression of IL-1β production (Fig. 3 A). The addition of rhIL-6, however, caused no significant change in the IL-1α a/IL-1β ratio in either group.

Effect of T Cell Cytokines on the Production of IL-1α a and IL-1β in Response to M. tuberculosis. It has also been shown that the lymphocyte production of IFN-γ and IL-4 can differentially modulate IL-1β and IL-1α a production (33). Our coculture system excluded the possibility of an obscuring effect of T cell cytokines by the sole use of PBMCs from PPD + individuals and a short culture duration. In fact, the production of IFN-γ was negligible in the M. tuberculosis-stimulated cultures (20 pg/ml) from these subjects. To investigate the possibility that T cell cytokines modulate M. tuberculosis-induced IL-1α a and IL-1β secretion, rhIFN-γ or rhIL-4 were added (0.1–10 ng/ml) to PBMCs. IL-4 caused a dose-dependent increase in both unstimulated and M. tuberculosis-stimulated IL-1α a production, which was most significant in the M. tuberculosis-stimulated

### Table I. Mean Fold Induction of the IL-1Rα and IL-1β Genes in Response to M. tuberculosis

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Number</th>
<th>Fold induction*</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1Rα</td>
<td>A2−</td>
<td>5</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>A2+</td>
<td>8</td>
<td>10.0</td>
</tr>
<tr>
<td>IL-1β</td>
<td>−511</td>
<td>3</td>
<td>25.9</td>
</tr>
<tr>
<td></td>
<td>+3953</td>
<td>10</td>
<td>46.5</td>
</tr>
</tbody>
</table>

|       | A2−    | 7              | 52.3  | 15.5–84.7 |
|       | A2+    | 6              | 29.3  | 11.3–47.5 |

Freshly isolated monocytes from 13 donors of differing genotypes were rested overnight and then stimulated for 4 h with M. tuberculosis at 1:1. Cytokine gene expression was quantitated by hybridization of 2 μg of the resultant RNA to [³²P]UTP-labeled complimentary RNA probes, including L32 and GAPDH as constitutively expressed “housekeeping” genes. The M. tuberculosis-induced fold increase in IL-1α a or IL-1β gene expression was calculated by dividing the band density in the presence of M. tuberculosis by the density in its absence.

*P values were 0.30 for IL-1Rα, 0.14 for IL-1β (−511), and 0.04 for IL-1β (+3953).
IL-1Ra A2+ group (P = 0.002 at 10 ng/ml). Furthermore, IL-4 also significantly decreased IL-1β production in M. tuberculosis-stimulated cells from both genotypes (P < 0.05 at 10 ng/ml). By comparison, IFN-γ led to a dose-dependent increase in M. tuberculosis-stimulated IL-1β production that was most marked in the IL-1R a A2+ group (P = 0.052 at 10 ng/ml). Thus, IFN-γ tended to increase IL-1β production in M. tuberculosis-stimulated cells without affecting IL-1R a production, whereas IL-4 increased IL-1R a production irrespective of genotype and also depressed IL-1β secretion. This differential effect was reflected in the mean M. tuberculosis-stimulated IL-1R a/IL-1β ratio, which increased in response to IL-4 even at the lowest dose of 0.1 ng/ml (P < 0.01, both groups combined). By comparison, higher doses of IFN-γ (1–10 ng/ml) were required to reduce the IL-1R a/IL-1β ratio significantly (Fig. 3 B).

Relationship between Polymorphism in IL-1R a and the Intracellular Growth of M. tuberculosis. We next investigated the effect of IL-1R a polymorphism on the rate of intracellular replication of M. tuberculosis. Monocytes from 22 donors (12 IL-1R a A2− and 10 IL-1R a A2+) were infected with M. tuberculosis at various multiplicities (1:1, 10:1, and 100:1 bacillus/cell) and then cultured in vitro for up to 240 h. Cell lysates were set up for M. tuberculosis CFU assay at 0, 24, 96, 168, and 240 h. Although there was interindividual variation in the establishment of initial infection, there was no significant difference between the IL-1R a A2− and IL-1R a A2+ groups. Logarithmic growth was established in 8 donors. The remainder showed either minimal or linear intracellular growth of mycobacteria only, with no difference between IL-1R a A2− and IL-1R a A2+ donors. In those donors in whom logarithmic growth did occur (5 IL-1R a A2− and 3 IL-1R a A2+), the doubling time of M. tuberculosis was estimated from the growth curve. Data from these individuals is shown in Table II. Intra- and interindividual differences did not appear to be related to the presence or absence of IL-1R a polymorphism (Table II).

Table II. Lack of Relationship between IL-1R a Polymorphism and the Intracellular Growth of M. tuberculosis In Vitro

<table>
<thead>
<tr>
<th>Donor</th>
<th>Genotype</th>
<th>Culture duration (h)</th>
<th>Doubling times at various multiplicities of infection by M. tuberculosis (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A1/A1</td>
<td>96</td>
<td>24 (2.47) 22 14</td>
</tr>
<tr>
<td>2</td>
<td>A1/A1</td>
<td>168</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td>A1/A1</td>
<td>240</td>
<td>39 (2.56) 32</td>
</tr>
<tr>
<td>4</td>
<td>A1/A1</td>
<td>240</td>
<td>38 (1.45)</td>
</tr>
<tr>
<td>5</td>
<td>A1/A1</td>
<td>240</td>
<td>42 28</td>
</tr>
<tr>
<td>6</td>
<td>A1/A2</td>
<td>96</td>
<td>27 (10.65) 15 13</td>
</tr>
<tr>
<td>7</td>
<td>A1/A2</td>
<td>168</td>
<td>38 28</td>
</tr>
<tr>
<td>8</td>
<td>A1/A2</td>
<td>240</td>
<td>53 (10.67) 29</td>
</tr>
</tbody>
</table>

Doubling times were estimated from the logarithmic growth curve in each case. The numbers in parentheses show the IL-1R a level in ng/ml 100,000 monocytes for the same individual at the corresponding multiplicity of infection.

—, Not tested.
The presence of the IL-1R α A2 allele. These data therefore contrast with the readily demonstrable increase in IL-1Rα secretion conferred by the A2 allele in the same donors (shown in parentheses in Table II). In each experiment, triplicate wells were also included to assess the effect of 2 μg/ml neutralizing antibody to IL-1Rα (and goat IgG isotype control). No consistent effect of these antibodies on intracellular growth was seen (data not shown).

IL-1β and IL-1Rα A2 allele frequency and G allele frequency in T tuberculosis patients and Tuberulin-reactive healthy control subjects. We next sought in vivo correlates by determination of the frequency of the IL-1β and IL-1Rα polymorphisms in patients with tuberculosis and healthy PPD-reactive control subjects in a pilot case-control analysis of Gujarati Asians in west London. This population is distinct and has a high incidence of tuberculosis with an excess of extrapulmonary forms. Individual alleles at each locus were in Hardy-Weinberg equilibrium. The IL-1β (−511) allele 1 was in linkage disequilibrium with IL-1β (+3953) allele 2 and vice-versa (P < 0.03). In addition, there was weaker linkage between IL-1Rα A2 and IL-1β (+3953) A2. No allele or genotype, singly or in combination, was associated with an increased risk of tuberculosis (Table III). We concluded that, in this population, these polymorphisms have little effect on susceptibility to tuberculosis per se.

The in vitro data indicated that the IL-1Rα A2−/IL-1β− (+3953) A1+ haplotype was associated with low IL-1Rα protein and gene expression and higher corresponding IL-1β values in vitro, implying a proinflammatory phenotype. This haplotype was more common in pleural disease, a form in which DTH is thought to be high, and was also associated with a moderately greater reaction to PPD in vivo. P values were calculated relative to the control group by Fisher’s exact test of probability, except for the comparison of median Mantoux diameter within the patient group, which was performed by the Mann-Whitney U test.

The number of patients with varying disease forms bearing the IL-1Rα A2−/IL-1β− (+3953) A1+ haplotype is compared to the number bearing other combinations. This haplotype was associated with low IL-1Rα protein and gene expression and higher corresponding IL-1β values in vitro, implying a proinflammatory phenotype. This haplotype was more common in pleural disease, a form in which DTH is thought to be high, and was also associated with a moderately greater reaction to PPD in vivo.

Discussion

We have investigated the effect of polymorphisms in the IL-1β and IL-1Rα genes on M. tuberculosis-stimulated cyto-
kin production in vitro and their relevance in patients with tuberculosis. When compared with healthy IL-1Ra A2– subjects, A2+ subjects as a group secreted nearly twice as much IL-1Ra in response to both laboratory-adapted and virulent M. tuberculosis, PPD, or LPS. The mean fold induction of IL-1Ra mRNA was also nearly twice that of IL-1Ra A2– subjects. The two polymorphisms in the IL-1β gene were not clearly associated with the level of M. tuberculosis–induced IL-1β production in vitro, although the IL-1β (+3953) A1+ haplotype was associated with significantly increased M. tuberculosis–induced expression of the IL-1β gene. The individual molar ratios of IL-1Ra/IL-1β, which determine the net effect of these cytokines in response to PPD and M. tuberculosis, were clearly higher in IL-1Ra A2+ subjects. Furthermore, the IL-1Ra/IL-1β ratios were affected by cytokines, as IL-4 upregulated IL-1Ra and downregulated IL-1β production. IL-10 greatly suppressed and IFN-γ moderately enhanced the production of IL-1β. In patients with tuberculosis, the proinflammatory IL-1Ra A2+/IL-1β (+3953) A1+ haplotype was unevenly distributed, being more common in patients with pleural disease and less common in those with extrapulmonary disease. A further finding, consistent with the in vitro observations, was that the IL-1Ra A2+ haplotype was associated with a reduced Mantoux response to PPD of M. tuberculosis: 60% of tuberculin-nonreactive patients were of this type.

Our study of IL-1RN gene expression indicates the early induction by M. tuberculosis of its mRNA together with IL-1β, IL-1α, TNF-α, and IL-6. Although IL-1RN A2 was associated with an increased induction of the IL-1RN gene, the exact mechanism of increased IL-1RN production requires further elucidation. Whereas the fold induction of IL-1β mRNA was higher than that of IL-1β and could also be related to both IL-1β polymorphisms, the amount of secreted IL-1β protein was much less. In addition, the IL-1β polymorphisms could not so readily be related to protein secretion. This observation is consistent with other data (36) and indicates a dominant influence of both posttranscriptional and posttranslational events on the secretion of IL-1β. Many cytokines can upregulate IL-1Ra expression in vitro (17). The production of IL-1Ra, however, was unaffected by antibody neutralization of IL-1β, IL-6, TGF-β, and TNF-α, suggesting that M. tuberculosis or its products induce the early production of large quantities of IL-1Ra by a direct mechanism.

IL-1β is involved in the early recruitment of inflammatory cells to M. tuberculosis– or PPD–induced granulomas (37–41). Submaximal occupancy of IL-1RIs can mediate the full biological effects of IL-1β, and as a consequence, it has been postulated that IL-1Ra needs to be present in a large molar excess in order to exert its antagonism (28). In tuberculosis, this condition would be best fulfilled in IL-1Ra and IL-1β subjects (Fig. 2); the IL-1Ra A2 allele was associated with reduced DTH (Fig. 4) and was lower in frequency in patients with pleural tuberculosis, consistent with the in vitro data and suggestive of biological significance. Antigen-specific lymphocytes are also necessary for the DTH reaction to proceed. In our experiments, IL-4 increased IL-1Ra secretion, particularly in stimulated cultures from IL-1Ra A2+ subjects (Fig. 3 B). The production of IL-4 in tuberculosis has been best demonstrated in T cell clones (42), but one study has also documented small amounts of antigen-specific secretion of IL-4 by PBM C (43). As cell-associated IL-4 is a stimulus for IL-1Ra, there is the possibility that relatively small amounts of IL-4 may greatly affect the IL-1Ra response (33). IFN-γ decreased and IL-10 increased the IL-1Ra/IL-1β ratio mainly through an effect on IL-1β secretion. Both IFN-γ and IL-10 are produced by PBM C and at disease sites in patients with tuberculosis (29, 44, 45). Our data therefore suggests that the polymorphism in the IL-1Ra gene may exert regulatory influence on cytokine circuits beyond its direct effect on IL-1Ra production.

There is both epidemiological and experimental evidence of a dissociation between DTH and protection from tuberculosis (46, 47). Our finding that IL-1Ra appears to influence DTH with minimal effect on either the intracellular growth of M. tuberculosis in vitro or disease susceptibility in the case-control study further suggests a basis for the dissociation between DTH and susceptibility. In addition to disease susceptibility, the degree of cutaneous reactivity to PPD after bacille Calmette-Guérin vaccination in both mono- and dizygotic twins and in siblings is also heritable (48, 49). Our in vitro data (Figs. 1 and 2) clearly support a functional basis for the observed association between reduced DTH and A2 of the IL-1RN gene. Although our case-control analysis was modest in size, there was a distinct difference in IL-1Ra A2+ frequency between patients with pleural and extrapulmonary tuberculosis, and this preliminary data encourages us to determine in larger studies whether this association is generalizable to other populations. As our data also support a heritable component in the quantitative skin response to PPD, another appropriate strategy would be to perform a genome-wide search, which may not only confirm the involvement of the IL-1 locus but also potentially identify loci of relevance to other infectious processes as well (50). As the frequency of the IL-1Ra A2 allele is approximately six times lower in Gambia (51) and also in Kenya (Wilkinson, R. J., and P. A. Zimmerman, unpublished...
We propose that the early recruitment and activation of in-genes can influence disease phenotype in tuberculosis (52). Expression supports the hitherto unproven concept that host genes can influence disease phenotype in tuberculosis (52). We propose that the early recruitment and activation of inflammatory cells by IL-1β to foci of tuberculous infection is in turn downregulated by IL-1R antagonists of IL-1R I (55) may also be a possible approach to modulation of immunopathologic cytokine circuits in tuberculosis.

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