Influence of Interleukin-1 Receptor Antagonist Gene Polymorphism on Disease

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Interleukin-1 receptor antagonist (IL-1RA) is a naturally occurring competitive inhibitor of interleukin-1 (IL-1)–induced proinflammatory activity. The IL-1RA gene is polymorphic, resulting in quantitative differences in both IL-1RA and IL-1β production. Persons homozygous for allele 2 of the IL-1RA gene (IL1RN*2) have a more prolonged and more severe proinflammatory immune response than persons with other IL-1RA genotypes. Thus, being IL1RN*2 homozygous might be beneficial when combating infectious agents or malignantly transformed cells, but it might be detrimental for those with chronic inflammatory conditions or who are pregnant. The IL1RN*2 phenotype is associated with ulcerative colitis and Crohn’s disease, lupus erythematosus, vulvar vestibulitis, and possibly with osteoporosis and coronary artery disease. IL1RN*2 homozygosity may also be associated with recurrent spontaneous abortion, preterm birth, and severity of preeclampsia. Conversely, there are negative associations between IL1RN*2 homozygosity and vaginal colonization with mycoplasmas, infection with human cytomegalovirus and Epstein-Barr virus, human immunodeficiency virus proliferation, and the occurrence of ovarian cancer.

It is becoming increasingly clear, as the DNA sequences of the human genome are being revealed, that many genes are polymorphic. In coding or noncoding regions of a specific gene, there may be either a single base pair substitution of one nucleotide for another or a variable number of repeats of a short, repetitive DNA sequence. These variations may influence the rate of gene transcription, the stability of the messenger RNA, or the quantity and activity of the resulting protein. Thus, the susceptibility or severity of a number of disorders will be influenced by possession of specific alleles of polymorphic genes.

One polymorphic gene that has received a great deal of research interest is the gene coding for the IL-1 receptor antagonist (IL-1RA). This gene is located on chromosome 2 in close proximity to the genes coding for IL-1α and L-1β [1, 2]. IL-1α and IL-1β are major inducers of proinflammatory immune responses. They both bind to the same IL-1 receptor on the surface of a variety of cells and initiate a cascade of events leading to recruitment and activation of macrophages and neutrophils, vascular dilation and fever, and a potent proinflammatory immune response [1]. The central role of the IL-1 system is protection against many different insults, ranging from microbial colonization to infection to malignant transformation. IL-1RA also binds to the same IL-1 receptor but does not initiate signal transduction. IL-1RA is thus a competitive inhibitor of IL-1 bioactivity. The relative levels of IL-1RA and IL-1 at an inflammatory site will thus determine whether a proinflammatory response will be initiated and persist or will be terminated [3]. Typically, the concentration of IL-1RA increases late during the course of an inflammatory event so that an induced acute inflammation can terminate and does not become chronic and damage healthy cells [4].

Experiments in mice either lacking (IL-1RA knock-out mice) or possessing extra copies (“overproducers”)
of the IL-1RA gene have further established the functions of this gene [5]. As expected, the survival of mice after endotoxin administration was directly proportional to the quantity of IL-1RA that they produced. An unanticipated essential role for IL-1RA in development was uncovered by the observation that IL-1RA knockout mice had lower body weights than did animals with normal or elevated IL-1RA production. The mechanism for this remains to be determined. It was also observed that IL-1RA appeared to stimulate IL-1 production in the presence of endotoxin. Serum IL-1 levels were highest in IL-1RA overproducers and lowest in the knockout mice. Perhaps in the presence of endotoxin, or other as yet undefined conditions, there is a coordinated expression of both the IL-1β and IL-1RA genes. Thus, depending on prevailing conditions, IL-1RA may inhibit IL-1 activity or upregulate IL-1 gene expression. This highlights the complex nature of interactions among cytokines.

### IL-1RA GENE POLYMORPHISM

In the second intron of the IL-1RA gene, there exists a tandem repeat sequence 86 base pairs in length [6]. The number of times this sequence is repeated in different persons varies from 2 to 6 (table 1). The frequency of the individual alleles varies among different ethnic or geographic populations, but allele 1 (IL1RN*1), containing 4 repeats, is always more common than allele 2 (IL1RN*2), containing 2 repeats. The remaining alleles, representing 3, 5, and 6 repeats, occur in <1% of most populations. In every population studied to date, most persons are either IL1RN*1 homozygous or IL1RN*1/IL1RN*2 heterozygotes. The prevalence of IL1RN*2 homozygotes is typically <10%. In black Africans and African American persons, the frequency of IL1RN*2 homozygotes is considerably lower than in the white population [7, 8].

Several studies have sought to demonstrate a relationship between the IL-1RA genotype and the concentrations of IL-1β and IL-1RA protein produced. Given the complexity of cytokine interactions and the diversity of the environment and the pathology of the populations studied, it is not surprising that IL1RN*2 has been associated with increased [9–12], decreased [13, 14], or similar [7, 8] IL-1RA protein levels as compared with the other alleles. In some studies where persons homozygous for IL1RN*2 had higher circulating IL-1RA levels than did persons with other genotypes, IL-1β levels were also elevated. This resulted in the lowest IL-1RA/IL-1β ratio and was associated with a heightened and prolonged proinflammatory immune response [11, 12, 15]. This observation parallels the association between elevated IL-1RA levels and increased IL-1β concentrations seen in endotoxin-treated mice [5].

### Table 1. IL-1 receptor antagonist gene polymorphism in intron 2.

<table>
<thead>
<tr>
<th>Allele</th>
<th>No. of repeats</th>
<th>Characteristics</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>Most common allele</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Associated with prolonged inflammation</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Rare</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>Rare</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>Rare</td>
</tr>
</tbody>
</table>

*Copies of an 86-bp repeat.

### IL-1RA PRODUCTION IN WOMEN AND MEN

IL-1RA is normally present in the circulation of healthy persons [16], whereas IL-1α and IL-1β are not typically detectable in the absence of disease or autoimmunity. A plausible explanation for this is that the circulating IL-1RA may function to prevent the unnecessary activation of proinflammatory immunity in response to minor nonpathogenic stimuli.

The production of IL-1RA, as well as IL-1, appears to differ between men and women [17]. The in vitro production of IL-1RA from isolated monocytes was significantly higher in women than in men. Furthermore, monocytes collected during the proliferative stage of the menstrual cycle released 2–3-fold greater levels of IL-1RA than did monocytes obtained from women in the luteal phase of the menstrual cycle. Similarly, IL-1RA levels in cervical mucus were lowest during the luteal phase in ovulating women [18]. This highlights the involvement of estrogen levels in regulating the IL-1 system. Primarily on the basis of these data, it has been suggested that the regulation of IL-1RA and IL-1 production may be fundamentally different in women than in men [19]. IL-1RA levels were shown to also be significantly higher in healthy 79-year-olds than in healthy 39-year-olds [20]. The significance of this observation to the decline in immunity with increasing age remains to be evaluated. Interestingly, levels of IL-1RA in amniotic fluids and urine of newborns were also shown to be significantly higher in female neonates than in males [21].

### IL-1RA AND PREGNANCY

IL-1β is a major factor in the initiation of infection-related preterm labor and delivery [22]. In mice, the injection of IL-1RA before IL-1β administration has been shown to prevent preterm birth [23]. Subsequently, IL-1RA was shown to be present in every human amniotic fluid examined at a concentration higher than that found in any other body fluid [24]. Studies in nonhuman primates confirmed the presence of IL-1RA in amniotic fluid and further demonstrated that its production was greatly enhanced after intrauterine infection or intra-amniotic IL-1β infusion [25]. The source of amniotic IL-
IL-1RA appeared to be fetal because IL-1RA levels became elevated in fetal plasma before increases in amniotic fluid concentrations [25]. The presence of IL-1RA in urine from human newborns [21] is further evidence for the fetal origin of this cytokine. IL-1RA in amniotic fluid, similar to its presence in serum samples, may function to prevent proinflammatory immune activation that could lead to a premature termination of pregnancy. Increased fetal production of IL-1RA in response to increases in IL-1 concentrations [25] offers further support for this hypothesis. Accordingly, women who produce low IL-1RA levels—or, more accurately, women possessing IL-1RA gene polymorphisms associated with low IL-1RA/IL-1β ratios—might be predicted to be at increased susceptibility to infection-related (proinflammatory cytokine-related) preterm labor and delivery. Our laboratory has, in fact, demonstrated that among Hispanic Americans, possession of IL1RN*2 by the fetus was associated with an increased prevalence of preterm birth [26]. This emphasizes that the genotype of the fetus, and not just that of the mother, must also be considered when analyzing genetic factors that might influence the outcome of pregnancy.

Conversely, IL-1RA has been shown to inhibit implantation of the early-stage embryo into the uterine decidua [27]. This is in keeping with studies describing an association between IL-1 production by preimplantation stage human embryos in vitro and subsequent successful implantation and development [28]. Thus, high IL-1RA producers—that is, those with the IL1RN*2 genotype—might be at a selective disadvantage at the beginning of pregnancy and at increased risk for early-stage pregnancy loss. A recent report has also suggested that Hispanic women who were IL1RN*2 homozygous had more severe preeclampsia than did preeclamptic women who possessed other IL1RN genotypes [29].

IL-1RA GENE POLYMORPHISM AND AUTOIMMUNE AND CHRONIC INFLAMMATORY DISORDERS

The majority of studies relating IL-1RA gene polymorphisms to disease have dealt with patients with autoimmune diseases or disorders associated with chronic inflammation. Associations have been reported between IL1RN*2 homozygosity and inflammatory bowel diseases [10, 13, 14], alopecia areata [30], psoriasis [31], lichen sclerosus [32], lupus erythematosus [33], vulvar vestibulitis [34], and multiple sclerosis [35]. The predominance of many of the above disorders in female subjects again highlights that IL-1RA regulation might differ between men and women. The low prevalence of inflammatory bowel diseases among blacks as opposed to whites also correlates with the rarity of IL1RN*2 in black persons [8].

The association between inflammatory bowel disease and IL1RN*2 homozygosity may not be universal but instead may be manifested only by certain ethnic groups. This relationship is apparent among American Jewish and Hispanic persons [14] and among persons from the United Kingdom [36], but not among Italians living in Italy [14] or persons studied in Hungary [37]. This is probably also true for other disorders, but this remains to be examined. Again, the complexity of cytokine interactions and regulatory mechanisms make it likely that polymorphisms in other genes also influence one’s final phenotype.

IL-1RA GENE POLYMORPHISM AND INFECTION

The association between IL1RN*2 and chronic inflammatory disorders suggests that persons with this allele have a more prolonged and more severe proinflammatory immune response than do persons with other IL-1RA genotypes. If this is indeed the case, than one would expect that the increased proinflammatory immunity of IL1RN*2 homozygotes might render these persons more efficient at combating microbial infection or colonization. Several studies suggest that this is in fact true. Vaginal colonization by the mycoplasmas Ureaplasma urealyticum and Mycoplasma hominis was reduced in women who were IL1RN*2 homozygotes [38]. Resistance to human cytomegalovirus [39] and Epstein-Barr virus [40] might also be increased in IL1RN*2 homozygotes.

Being IL1RN*2 homozygous may also be beneficial for women in the immune defense against HIV replication [41]. Among Brazilian women infected with HIV who were at similar stages in their disease, those who were IL1RN*2 homozygous had significantly lower levels of HIV-1 RNA in their circulation than did women with other IL-1RA genotypes. There was no relation between IL-1RA alleles and circulating CD4+ T lymphocyte concentrations.

IL-1RA polymorphism has also recently been shown to influence the sequelae to Mycobacterium tuberculosis infection [12]. Possession of IL1RN*2 was associated with increased production of IL-1RA messenger RNA and protein and a higher IL-1RA/IL-1β ratio in response to M. tuberculosis. This appears to be opposite what is observed in autoimmune diseases where there typically is a lowered IL-1RA/IL-1β ratio in IL1RN*2 homozygotes. Perhaps M. tuberculosis can selectively downregulate IL-1β gene activity in these persons. The IL-1RN*2 genotype also correlated with a reduced delayed type hypersensitivity response to purified protein derivative (PPD) and was inversely related to the development of pleural tuberculosis. Thus, possession of a particular IL-1RA genotype may influence the course of tuberculosis.

IL-1RA POLYMORPHISM AND CANCER

The concentration of IL-1RA is elevated in serum samples of women with gynecologic cancers, as compared with women
with benign conditions or healthy control women [42]. The significance of this is undetermined and is undoubtedly the culmination of a multiplicity of events. If the IL-1 system is a key component of the immune defense against any of the gynecologic cancers, then it might be expected that women who were IL1RN*2 homozygous might have a genetic advantage in cancer prevention because this genotype is associated with prolonged and more severe inflammatory reactions. In a study of 310 women who were seen for a possible gynecologic malignancy and 343 healthy control women, the prevalence of the IL1RN*2 homozygous genotype was 8.5% in controls, 9.6% in 83 women with benign conditions, and 0% in 46 women with ovarian cancer (P = .03) (I. Korneeva et al., in preparation). Conversely, there was no relation between IL-1RA genotype and endometrial cancer. The data suggested that the IL1RN*2 genotype might be associated with an increased capacity for immune surveillance specifically against malignant transformation in ovarian epithelial cells.

**IL-1RA POLYMORPHISM AND OSTEOPOROSIS**

The regulation of postmenopausal bone loss is another area whose consequences vary with the IL-1RA genotype. Osteoporosis, a disorder characterized by decreasing bone mass and a subsequent increased susceptibility to fractures, is progressive in postmenopausal women. Studies in rats whose ovaries were removed have demonstrated that the loss of estrogen function is accompanied by a rapid increase in bone cell turnover and decreased bone density. These changes were prevented by administration of exogenous estrogen. Administration of exogenous IL-1RA also markedly decreased bone resorption, but only in ovariectomized animals. This indicated that IL-1RA inhibited estrogen-dependent bone loss [43]. Other studies have demonstrated that IL-1 is a potent stimulator of bone resorption [44]. More recent studies have suggested that the mechanism whereby estrogen prevents bone loss may be via the induction of IL-1RA [45, 46].

Several investigators have examined the relationship between IL-1RA gene polymorphism and osteoporosis. Two studies concluded that the IL-1RA genotype influenced development of osteoporosis [47, 48], and a third study found no such relationship [49]. In the first study of 108 postmenopausal women [47], after adjustment for confounding variables such as age, weight, and baseline bone mineral density, carriage of at least one copy of the IL1RN*2 allele was associated with a significant reduction in bone loss of the spine compared with women who lacked this allele. The second study [48] identified a relationship between being homozygous for the IL1RN*1 allele and an increased risk of osteoporotic fractures in the 389 patients studied. Bone mineral density was unrelated to IL-1RA genotype in the 286 subjects who comprised the third study [49]. The fact that the third study was performed on Hungarian women but women from England and Denmark were analyzed in the two former studies might account for the different results, again highlighting the necessity of defining the population studied.

**IL-1RA POLYMORPHISMS AND CORONARY ARTERY DISEASE**

A relationship between IL-1RA genotype and coronary artery disease has been reported in a small number of investigations. In a British study, persons with single-vessel coronary artery disease had a significantly higher prevalence of IL1RN*2 than did healthy controls. Somewhat surprisingly, the distribution of the IL-1RA alleles in those persons with multivessel coronary disease was similar to that of the control population. The authors concluded that different genetic components might be responsible for single artery and multiple artery coronary disease [50]. The increased proinflammatory immune response associated with IL1RN*2 thus might increase susceptibility to arterial wall damage. However, a second, very large study of 1850 persons found that carriers of IL1RN*2 were at significantly lower risk for restenosis of their coronary arteries after stenting compared with persons with other IL-1RA alleles [51]. A third study found a nonstatistically significant tendency (P = .20) for an increased IL1RN*2 frequency among persons aged <40 years who experienced myocardial infarction [52].

**A NOTE OF CAUTION**

A potential shortcoming in studies that examine associations between alleles of a polymorphic gene and a particular disease is the possibility of linkage disequilibrium. The allele being studied might be linked to another as yet unidentified gene, and this second (or third) gene may actually be responsible for the observed phenotypic difference. The apparent relationship between IL-1RN*2 and such a wide range of disorders raises the possibility that another nearby gene might be responsible for some of these associations.

**MODULATION OF IL-1RA CONCENTRATIONS**

Exogenous IL-1RA has been administered with varying degrees of success in attempts to alleviate numerous experimental animal models of disease [19]. In humans, high levels of exogenous IL-1RA were shown to be without detectable toxicity [53]. However, utilization of exogenous IL-1RA to combat the extensive proinflammatory immune response that occurs in sepsis has been unsuccessful [19]. The effects of modifying the levels of IL-1RA on the severity of chronic inflammatory disease such as rheumatoid arthritis and inflammatory bowel disease are currently being investigated. Promising results in the treat-
mment of rheumatoid arthritis have been reported [54, 55]. Future studies that use exogenous IL-1RA or IL-1RA inducers to treat various disorders should take into consideration whether or not the persons tested are homozygous for IL1RN*2.

References

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