A functional interleukin-10 mutation in Dutch patients with Crohn’s disease

K. van der Linde a, P.P.C. Boor b, A.A. van Bodegraven c, D.J. de Jong d, J.B.A. Crusius e, T.H.J. Naber d, E.J. Kuipers a, J.H.P. Wilson b, F.W.M. de Rooij b,∗

a Department of Gastroenterology and Hepatology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands
b Department of Internal Medicine, Room Bd 299, Erasmus MC, University Medical Center, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands
c Department of Gastroenterology, VU University Medical Centre, Amsterdam, The Netherlands
d Department of Gastroenterology and Hepatology, University Medical Centre St. Radboud, Nijmegen, The Netherlands
e Laboratory for Immunogenetics, VU University Medical Centre, Amsterdam, The Netherlands

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Abstract

Background and aims. Interleukin-10 (IL-10) is an anti-inflammatory and immunomodulatory cytokine. IL-10 deficient mice are prone to develop chronic colitis. Administration of recombinant human interleukin-10 has been proposed to have a beneficial effect in a subgroup of patients with Crohn’s disease. Recently, we found an interleukin-10 Gly15Arg mutation in a family with Crohn’s disease which is associated with reduced interleukin-10 secretion by in vitro stimulated monocytes and lymphocytes. We hypothesised that this interleukin-10 mutation plays a role in maintaining the inflammatory process in Crohn’s disease in some families.

Patients and methods. We evaluated interleukin-10 Gly15Arg in 379 patients with Crohn’s disease, and 75 unrelated healthy controls. Also, first degree family members of interleukin-10 Gly15Arg carriers were evaluated. Additionally, mutation carriers and their relatives were evaluated for CARD15 R702W, G908R, and 1007fs.

Results. Two patients with Crohn’s disease were heterozygous for the interleukin-10 Gly15Arg mutation. No homozygotes were found. The Gly15Arg mutation was not observed in the controls. In first degree family members of the Crohn’s disease-affected interleukin-10 Gly15Arg carriers, the mutation was found in Crohn’s disease-affected as well as in their apparently healthy individuals. All family members carried one or two CARD15 mutation(s).

Conclusion. The interleukin-10 Gly15Arg mutation is rare in patients with Crohn’s disease, and is not associated with the disease in the Netherlands.

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Keywords: Crohn’s disease; Inflammatory bowel disease; Interleukin-10; Mutation

1. Introduction

Interleukin-10 (IL–10) is considered to be an important cytokine for immune modulation in the gut. It has anti-inflammatory and immunomodulatory effects, and is considered to play a role in inflammatory bowel disease (IBD). This hypothesis is supported by the observation that IL–10 gene knockout mice develop chronic enterocolitis resembling Crohn’s disease (CD) [1]. IL–10 influences the expression of a variety of cytokines, soluble mediators, and cell surface molecules produced by activated monocytes and macrophages. IL–10 can down-regulate the production of IL–1 and tumour necrosis factor alpha (TNFα), which are first line cytokines in a Th1-driven inflammatory response [2]. Subcutaneously administered IL–10 has been evaluated in four clinical trials with CD-affected patients, with inconsistent results [3–6]. It has been suggested
that a subgroup of CD patients might benefit from IL-10 ad-
ministration [4].

Recently, we found an IL-10 single nucleotide gene al-
teration at position 43 in exon 1 (G → A) encoding for a
Gly15Arg substitution in a family with multiple CD-affected
members. The nucleotide substitution was associated with a
reduced IL-10 protein secretion by in vitro stimulated mono-
cytes and lymphocytes of IBD patients [7]. Based on these
findings we hypothesised that the IL-10 Gly15Arg mutation
may play a role in the pathogenesis of IBD in this family, and
that IL-10 administration could be useful in treating active
CD in these patients [7].

CD is considered to be a polygenic disease with different
genes contributing to susceptibility in different families and
giving rise to differences in response to anti-inflammatory
agents [4]. Recently, three coding polymorphisms R702W,
G908R, and 1007fs in the CARD15 gene were shown to be
independently associated with CD [8,9]. As the IL-10
Gly15Arg also appeared to be a coding nucleotide variation
and therefore may have potential therapeutic implications, we
evaluated the prevalence of this mutation in a large group of
CD-affected patients with or without a positive family history
of IBD.

2. Patients and methods

2.1. Patients and materials

Patients with CD of Dutch Caucasian origin were re-
cruited from three Dutch University referral centres. Patients
were classified as familial CD if one or more first, second or
third degree relatives were known to have IBD. If the family
history was negative or not clear, patients were considered
sporadic CD or CD patients with an unknown family history.
The diagnosis of CD was verified by each centre according to
standard criteria [10]. The age of the CD-affected partic-
ipants was calculated on the date of entry, i.e. July 1st 2002.
Initially, only the proband from a family was studied. Family
members of selected participants were also subsequently
examined.

Seventy-five healthy Caucasian controls were included.
These controls were recruited at the Erasmus MC, University
Medical Center, and the reports have been published previ-
ously [7]. Genomic DNA was isolated from whole peripheral venous
blood using standard techniques.

The study was approved by the Ethics Committee of the
Erasmus MC, University Medical Center, Rotterdam. All par-
ticipants provided written informed consent.

2.2. IL-10 Gly15Arg mutation analysis

Exon 1 of the IL-10 gene was amplified using the forward
primer 5′-AGAGGCTCTCCCTGAGCTTAC-3′ and reverse
primer 5′-TGTGGGGATGAGTTGGAG-3′ resulting

in a polymerase chain reaction (PCR) fragment of 386 bp.
The prevalence of the IL-10*G/A point mutation at base
position 43 was evaluated with the restriction enzyme Mae
I (Boehringer Mannheim, Almere, The Netherlands). After
digestion with 1 unit of Mae I at 45 °C for 4 h, the PCR
fragment was analysed on a 2% agarose electrophoresis
gel. Double bands were visualised with ethidium bromide
staining. Wild-type PCR products resulted in fragments of 60
and 326 bp, while mutant PCR products gave fragments
with a length of 60, 152 and 174 bp. Positive samples
representing the IL-10 Gly15Arg mutation were confirmed
by sequencing the 386 bp PCR fragments, using the for-
ward primer 5′-AGAGGCTCTCCCTGAGCTTAC-3′ and a
BigDye Terminator sequencing kit (Applied Biosystems,
Nieuwerkerk aan de IJssel, The Netherlands). All DNA
sequences were performed using an ABI 310 Genetic
Analyser (Applied Biosystems) supported by Genescan
(version 2.1.1) and Genotyper (version 2.1) software
programs.

2.3. IL-10 haplotyping

Microsatellite markers IL-10.G and IL-10.R were used for
haplotyping IL-10 alleles. Both microsatellites have been
evaluated in healthy Caucasian individuals by Eskdale et al.
[11]. The IL-10.G marker is highly polymorphic with a het-
erozygosity of around 72%. It is located in the promoter of
the IL-10 gene [11]. The IL-10.R marker is less informative,
having a heterozygosity of 50% [12]. PCR conditions were
used as published in the literature with minor changes. An
ABI 310 Genetic Analyser (Applied Biosystems) was used
for DNA sequencing.

2.4. CARD15 mutation analysis

CARD15 R702W and G908R were determined by means
of restriction enzyme analysis. First, the DNA regions of in-
terest containing the alteration were amplified by PCR. For
R702W (2104C → T in exon 4) the forward primer 5′-CAG
CTG GGC AGC TTG GCT GC-3′ and the reverse primer
5′-CAT GGC ATG CAC GCT TTG GG-3′ were used. The
448 bp was purified by phenol extraction followed by ethanol
precipitation, after which the PCR fragment was digested
with 8 units of Hpa II (New England BioLabs, Beverly, USA)
for 4 h at 37 °C. Bands were visualised after separation on
a 2% agarose gel containing ethidium bromide. The wild-
type exon 4 resulted in fragments of 29, 54, 76 and 289 bp,
whereas the minor allele resulted in fragments of 29, 130,
and 289 bp. The G908R (2722G → C in exon 8) alteration
was amplified using the forward primer 5′-CAC TTG GCT
GGG ACC AGG AG-3′ and reverse primer 5′-ACT CCA
TTG CCT AAC ATG GCT G-3′. The 363 bp PCR product
was digested immediately with 4 units HpaI I (New Eng-
land BioLabs) for 3 h at 37 °C followed by separation on a
2% agarose gel containing ethidium bromide. It resulted in
one fragment of 363 bp in case of the wild-type and in 168
and 195 bp fragments in case of the rare allele of the G908R polymorphism.
CARD15 1007fs (3020insC in exon 11) was determined by means of an allele-specific PCR reaction, followed by separation of PCR products on an agarose gel as described previously [9,13].

When participants were positive for CARD15 R702W, G908R or 1007fs, sequencing of the corresponding PCR product was performed on an ABI 310 genetic analyser (Applied Biosystems) to confirm the alteration.

2.5. Statistical analysis
Statistical analysis was performed by comparing the allele frequencies of the mutation in patients with CD and controls, using Fisher’s exact test. The genotype frequencies in controls were tested for Hardy–Weinberg equilibrium (HWE) proportions. The analyses were conducted with SPSS software version 9.

3. Results
3.1. Patients
Initially, 79 probands from separate families with CD were studied. Subsequently, 300 CD patients with no or an unknown family history were investigated, giving a total of 379 patients with CD including 134 males and 245 females. Their mean age was 40.1 years (range: 18.7–81.6 years).

3.2. Prevalence of IL-10 Gly15Arg mutation
Two CD patients (0.5%) from two different multiple IBD-affected families were found to be heterozygous for the IL-10 Gly15Arg mutation (Fig. 1). Both probands were from one centre (Erasmus MC, University Medical Center). Homozygosity for the Gly15Arg mutation was not found. All other CD-affected patients and all 75 healthy controls carried the wild-type allele of the IL-10 gene.

3.3. Mutation analysis in family members of IL-10 Gly15Arg mutation carriers
First degree family members of the two CD-affected IL-10 Gly15Arg mutation carriers (probands) were evaluated for this mutation. In both families IL-10 haplotypes were evaluated as well. The results are summarised in Fig. 1. The reports of one family has been published recently [7]. In family 2, in addition to the proband a niece of the probands’ father had CD. She was evaluated as well, and did not have the Gly15Arg mutation.

One CD-affected sibling and several non-IBD-affected family members were carriers of the IL-10 Gly15Arg mutation. In both families, the mutation was present on the haplotype IL-10.43*A-IL-10.G-131 bp-IL-10.R-110 bp. Therefore, it is possible that these families have a common ancestor.

3.4. Clinical characteristics of two families with the IL-10 Gly15Arg mutation
3.4.1. Family 1
This family has been described in detail recently [7]. In summary, the proband (born in 1963) has been known to be with CD since 1979. The disease is mainly located in the small bowel. He was treated with salazopyrine and steroids, and later budesonide. He underwent several resections for therapy resistance and stenotic lesions. The disease was complicated by uveitis and sacroiliitis. One female CD-
Table 1

<table>
<thead>
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<th>Study</th>
<th>CD (c)</th>
<th>Cardiovascular disease (c)</th>
<th>Healthy controls (c)</th>
</tr>
</thead>
<tbody>
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<td>Klein et al. [21]</td>
<td>2</td>
<td>140</td>
<td>–</td>
</tr>
<tr>
<td>Donger et al. [22]</td>
<td>–</td>
<td>–</td>
<td>7</td>
</tr>
<tr>
<td>This study</td>
<td>2</td>
<td>377</td>
<td>–</td>
</tr>
<tr>
<td>Totalb</td>
<td>4</td>
<td>517</td>
<td>7</td>
</tr>
<tr>
<td>Prevalence (%)b</td>
<td>0.8</td>
<td>99.2</td>
<td>99.9</td>
</tr>
</tbody>
</table>

a All IL-10 Gly15Arg positive individuals are heterozygous.

b Statistical analysis of the combined data using Fisher’s exact test showed no association between IL-10 Gly15Arg and CD.
In this study, 75 unrelated healthy controls were analysed for the IL-10 Gly15Arg mutation. No carriage was found. We concluded that the IL-10 Gly15Arg mutation is not associated with CD. Ideally, to evaluate the possibility of association, a much larger control group is necessary. However, as Klein et al. and Donger et al. [21,22] showed a very low prevalence of IL-10 Gly15Arg in a large set of control, enlarging our control group is unlikely to change the conclusion of this study.

As healthy carriers of the mutation were found in both families, the prevalence of the IL-10 Gly15Arg mutation is not by itself sufficient to cause CD in these specific families. We speculate that in a subgroup of CD patients, the reduction in IL-10 production together with other yet unidentified predisposing genes involved in the immune response, may impair the capacity to appropriately overcome or down-regulate the inflammatory process in the gut. The finding that carriage of the mutation is not associated with CD, and that the finding that within a family with two CD-affected relatives only one carries the mutation, does not exclude a pathogenetic role for this functional mutation. Similar disparities in association have for instance been reported for the CARD15 mutations in CD [8,9,13,23]. Moreover, there might be an epistatic interaction between IL-10 and CARD15 as in both families CD-affected individuals were found with both the IL-10 Gly15Arg as well as one or two CARD15 mutations.

In conclusion, the prevalence of the IL-10 Gly15Arg mutation is very low (less than 1%) and is only found in a small number of individuals with CD. However, additional gene polymorphisms and/or triggering events are necessary to result in clinical manifest CD.

Conflict of interest statement
None declared.

List of abbreviations
CD, Crohn’s disease; IBD, inflammatory bowel disease; IL-10, interleukin-10; PCR, polymerase chain reaction; TNFα, tumour necrosis factor alpha.

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References

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