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Semiautomated and simultaneous analysis of the interleukin-10 gene microsatellites IL-10G and IL-10R by fluorescence-based polymerase chain reaction reveals significant differences in allele distributions between Caucasians (Germany) and Africans (Gabon)

Abstract

Interleukin-10 (IL-10) is an important immunoregulatory cytokine influencing many aspects of the adaptive and inflammatory immune reponse. Two dinucleotide repeats have been identified in the 5'-UTR of IL-10 and shown to be useful genetic markers in several diseases. A simple, two-colour fluorescence assay was developed for determination of microsatellite fragment length by an automatic sequencer. Using this method polymorphisms at the IL-10G and IL-10R loci of the 5' flanking region of the IL-10 gene can be identified simultaneously. A unified standard nomenclature was applied to the known IL-10G and IL-10R microsatellites. The multiplex PCR approach was used to compare the allele frequencies in two independent donor groups from Germany (Caucasian), comprising 112 and 106 cases, respectively, and one group from Gabon (African) including 91 donors. Significant differences in the allele distribution were found. Both Caucasian populations tested showed no significant differences in their allele and genotype distribution. Whereas in Africans, allele IL-10G25 is rare at 3% compared to 21% in Caucasian, alleles IL-10G22 and G23 are more prevalent in Africans than in Caucasians (22% versus 10% and 26% versus 7%, respectively). Within the IL-10R locus, the allele R13 was observed at 88% in the African group compared to 69% in Caucasians. These data may help immunogenetic studies in diseases, where IL-10 is thought to be deregulated.

Full text

INTRODUCTION

Interleukin-10 (IL-10) is an important immunoregulatory cytokine [1, 2]. The ability of IL-10 to block the activation of cytokine synthesis and several accessory cell functions renders this cytokine a potent suppressor of the effector functions of monocytes/macrophages, T cells and NK cells. In addition, IL-10 may contribute to the regulation of proliferation and differentiation of B cells [3-5]. The expression of IL-10 appears to be tightly regulated since the levels of constitutive expression in normal cells are extremely low.

IL-10 is involved in the regulation of inflammatory reponses, in the pathology of human autoimmune disease and transplantation complications [6-14]. IL-10 also plays an important role in the development of infectious diseases, such as

Plasmodium falciparum malaria or Epstein-Barr virus-associated tumors [4, 15-23]. The ability of IL-10 to induce T cell anergy and to downregulate MHC class II expression may also be important in its apparent contribution to tumor-related immunosuppression [24].

Recent characterization of the promoter and 5' flanking regions has demonstrated the presence of positive and negative regulatory elements of the promoter [25, 26]. In addition, the definition of two dinucleotide tandem repeat elements immediately upstream of the gene, as well as a number of single nucleotide polymorphisms (SNPs), have shown that there is considerable polymorphism directly associated with the human IL-10 gene [27-30].

The analysis of short tandem repeat (STR) loci by PCR methods has proven to be informative for a variety of applications [31, 32]. Dinucleotide repeat loci $(CA)_N$ as found in the IL-10 gene locus, are very abundant and are routinely used as markers in linkage studies and for building genetic maps. So far, 13 IL-10G alleles spanning from 16 to 28 CA dinucleotide repeats and 5 IL-10R alleles characterized by 12 to 16 repeats have been described [27, 38]. The most frequent IL-10G allele is G9 and for IL-10R it is R2. IL-10G9 consists of 21 CA dinucleotide repeats, IL-10R2 has 13 CA repeats.

In recent studies, striking evidence for differences between individuals in their ability to produce IL-10 was demonstrated [33]. This was shown to be attributable to the structure of the IL-10 gene locus, as defined by haplotypes containing alleles from the two microsatellite loci.

Thus, a simple method for analysis of these microsatellites, within the IL-10 gene, is important not only for answering scientific questions, but also for routine typing of alleles in corresponding disease groups. In order to define IL-10 haplotypes associated with the known microsatellites using a one tube PCR-reaction, a new semiautomated multiplex method was created. This method was developed to analyse different populations from Europe (North-Germany (Cologne) & South-Germany (Stuttgart) and Africa (Gabon, Lambarene).

MATERIAL AND METHODS

DNA Samples

All samples were taken with no regard to sex or age, and donors were free of any chronic diseases.

The DNA from donors from Stuttgart was isolated from buffy coats obtained from the blood donation centre of Stuttgart. The DNA from the Cologne population was isolated from volunteers of the University Hospital Cologne.

The DNA from the African population was from Lambarene/Gabon as described previously [34].

Isolation of DNA

DNA was purified from 200 mul of whole blood using the Qiagen blood kit according to the manufacturer's instructions (Qiagen, Hilden, Germany).

PCR, electrophoresis and fragment analysis

The IL-10G and IL-10R microsatellites were analysed using fluorescence-labeled primers. For duplex amplification of IL-10 microsatellites, a mixture of the four primers IL-10G23/IL-10G12-FAM and IL-10R11-HEX/IL-10R21 at 20 ng/mul each was used (all primers were purchased from Eurogentec, Seraine, Belgium) (Table 1) [26]. PCR was performed on a Biometra UNO-II termocycler using Platinum Taq (Gibco-BRL) with the following cycling conditions: 96° C 30 sec, 66° C 30 sec, 72° C 30 sec for 25 cycles and 96° C 30 sec, 62° C 30 sec, 72° C 30 sec for additional 10 cycles. A third primer set was introduced into the multiplex PCR (TGCA, TGCA-HEX) spanning a fragment of 141 bp. The cycle conditions for the triplex PCR were modified as following: 96° C 30 sec, 66° C 30 sec, 72° C 30 sec for 25 cycles followed by 10 cycles at 96° C 30 sec, 62° C 15 sec, 72° C 15 sec. All PCR reactions were terminated by a final extention step for 60 min at 72° C. In every PCR-cycle, positive controls having a known number of CA-repeats (pGL2-E-PIL103, $\{CA\}_{21}$), and a water control were included. The sequence <GTT TCT G> was used at the 5'-end of some primers (IL-10G23, IL-10G12-FAM and IL-10R21) to force the A-tailing typical of Taq-polymerase.

The quality and amount of PCR products were estimated on a 3% NuSieve agarose (FMC) gel in 0.5 x TBE using ethidium bromide to visualize DNA and a 100 bp length standard (Gibco BRL, Breda, The Netherlands). The samples were then mixed in the appropriate dilution with a loading buffer containing bromphenol

blue, formamide and a CXR-labeled length standard (Promega, Woods, Madison, USA). The length standard was visualized as red signals, has a band spacing of 20 bp in the range between 60 and 200 bp and a spacing of 25 bp between 200 and 400 bp. 2 mul of the diluted microsatellite amplimer/loading buffer mixture was analysed on a 4.5% denaturing acrylamide gel as recommended by the manufacturer of the sequencer (ABI377: Applied Biosystems, Perkin-Elmer, Foster City, CA, USA). For detection of the FAM-labeled (blue colour) and HEX-labeled (green colour) PCR products, the filter set D was used. Data were collected and processed using GENESCAN software according to the manufacturer's instructions. The electropherograms generated from the processed gel images were in addition manually assessed for accurate size determination and the estimation of quality of the allelic peaks. In further analysis, samples were only included where the intensity of the PCR-products gave relative fluorescence units between 500 and 4,000 for the main peak.

The fragment length data were analysed using Microsoft Excel and afterwards by GENEPOP software [40]. The statistical significance of Hardy-Weinberg differences, intergroup differences of alleles, and genotype frequencies were determined using exact test procedures by GENEPOP software.

Allele designation

In accordance with the recommendations of the DNA Commission of the International Society for Forensic Haemogenetics for human STR systems, allele designation is based on the number of repeating units [35]. Following these rules, allele IL-10G9 described before and consisting of 21 CA-repeating units was renamed IL-10G21, and IL-10R2 with $(CA)_{13}$ was designated as IL-10R13 [27, 28].

Whole genome PCR

A whole genome PCR of DNA samples was performed in order to multiply the amount of DNA available for further downstream applications. DNA samples were multiplied by different cycles of primer extension using either a collection of 15-base oligonucleotides or 10-base oligonucleotides (PEP PCR). Primers were purchased from Birsner & Grob-Biotech GmbH (Freiburg, Germany). Any of the four possible bases was present at each position. Therefore, this corresponds to a theoretically mixture of 4^{15} (1×10^9) sequences [36]. Samples

were amplified in the following mixture in a total volume of 40 mul: 0.17 mM dNTPs, 10 x PCR buffer A (Promega), 33.3 muM random-primer, 2.5 mM $MgCl_2$, and 1 mul Taq polymerase for buffer A (5U/mul) (Promega). After an intitial denaturation at 95° C for 10 min, the *Taq* polymerase was added at 80° C, followed by 2 min at 37° C and 4 min at 55° C. Then, 49 primer extension cycles were carried out. Each cycle consisted of 1 min denaturation at 95° C, 2 min annealing at 37° C and 4 min incubation at 55° C for polymerase extension.

Specific gene analysis

We subjected aliquots of the PEP amplified DNA samples to tests for the presence of specific DNA sequences. A total of 3 independent loci were used for verification of whole genome amplification (i) mannose-binding lectin (MBL), (ii) intercellular adhesion molecule-1 (ICAM-1) and (iii) IL-10. To determine whether PEP contributes to a higher rate of PCR errors, the exact sequence was determined by automatic sequencing using an ABI sequencer 373. All PCRs for ICAM-1, MBL and PEP were performed on a Hybaid (Omnigen), according to standard procedures.

The whole genome PCR for the IL-10 microsatellites was performed using the same conditions and primers as described in Material and methods (PCR, electrophoresis, and fragment analysis).

RESULTS AND DISCUSSION

Semiautomated multiplex analysis of IL-10G and IL-10R microsatellites

The IL-10G and IL-10R microsatellites were analysed using fluorescence-labeled primers. Data were collected and processed using GENESCAN software according to the manufacturer's instructions. The electropherograms generated from the processed gel images were, in addition, manually assessed for accurate size determination and the quality control of data peaks. In further analysis, samples yielding main peaks with relative fluorescence signals between 500 and 4,000 units were included.

The PCR conditions were optimized using a cloned fragment of the 5' flanking region of the IL-10 gene [26].

In Figure 1, typical examples of a multiplex PCR using primer IL-10G12-FAM/IL-10G23 and IL-10R11-HEX/IL-10R21 are shown. The amplification of a homozygous sample in IL-10G and IL-10R is shown in Figure 1A (IL-10G21, IL-10R14), a sample heterozygous for IL-10G and homozygous for IL-10R (IL-10G21/25, IL-10R13) is demonstrated in Figure 1B and a sample heterozygous for IL-10G and heterozygous for IL-10R (IL-10G21/23, IL-10R13/14)) can be seen in Figure 1C.

In addition, a comparison of different polymerases for PCR was performed. Conventional Taq polymerase was compared with proof-reading polymerases and hot start Taq polymerase (data not shown). No significant differences were found.

At -2,471 from the transcriptional start site of the IL-10 gene, the sequence taTGTGTGTGTGTGTGTatttaCACACACAtc was decribed [28]. Thus, to test the polymorphic nature of this region, a third primer set was introduced into the multiplex PCR, spanning a fragment of 141bp containing this reversed, dinucleotide repeat consisting of TG/CA dinucleotide repeats (Figure 2A). The additional primer pair did not influence the amplification of the other two loci. A typical sample of a three-loci PCR, analysed on an acrylamide gel is shown in Figure 2B. This reversed dinucleotide repeat was called IL-10IR.

PCR-amplified STR loci were analysed by gel electrophoresis and visualized as bands on gels, or were represented by peaks on electropherograms. All of these analytical methods detected a particular type of PCR product that is produced by amplification of STR loci. This product, which differs in size from the main allele by multiples of the repeat unit size, has been referred to in the literature as a shadow band, DNA polymerase slippage product or stutter band [31, 32, 37]. In the case of dinucleotide repeat loci, the most prevalent stutter band is generally two bases shorter than the main allele band, with additional stutter bands shorter by four and six bases. Because of the multiband pattern for each allele, interpretation of dinucleotide repeat loci is sometimes complicated when the two alleles from a single individual are of similar in size.

So far, PCR has been performed for the detection of microsatellites using radioisotope-labeled primers, with autoradiography following electrophoresis [31, 32, 37]. Since in these assays slippages are often difficult to distinguish from PCR errors, reproducible characterization of microsatellites is sometimes difficult to obtain in a simple fashion. No methods for eliminating the stutter

bands in dinucleotide repeat loci have yet been identified. However, the proportion of bands is quite reproducible for each of the different alleles and so general rules for interpretation can be invoked. By using fluorescence-based detection of PCR products, the estimation of the proportion of stutter bands for each allele can be determined.

The amplification using IL-10R primers led to a dominant PCR product with a - 2 bp stutter band of no more than 25% intensity/peak height compared to the major allele-associated band (Figure 1).

In the case of the IL-10G allele, three major slippage bands were identified (Figure 1A). The - 2 bp stutter band was at about 75% intensity/peak height compared to the major allele-associated band (Figure 1C). When both IL-10G alleles differ by only 1 CA repeat on the electropherogram, the second major allele peak is only about half the major allele peak of the shorter allele. Until methods to eliminate stutter band formation during PCR are available, interpretation of results can be improved by careful characterization and quantification of stutter bands for all alleles at the STR loci of interest.

Whole genome preamplified DNA used in the semiautomated mulitplex analysis of IL-10G and IL-10R microsatellites

Sometimes, only limited DNA material may be available in field of clinical studies. Therefore, DNA was preamplified and subjected to a whole genome PCR in order to multiply the amount of DNA available for further downstream applications (PEP PCR). A total of 3 independent loci were used for verification of whole genome amplification (i) mannose-binding lectin (MBL), (ii) intercellular adhesion molecule-1 (ICAM-1) and (iii) IL-10 (data for MBL and ICAM1 not shown).

Tests were performed to see whether the developed multiplex approach can be also used with preamplified DNA. As shown in Figure 3A, from analysing the amplimers on an agarose gel, it is obvious that the preamplification of genomic DNA with random primers is associated with an increased background, using the three primer pair amplification (for example ethidium bromide staining in the slots), and decreased amounts of amplimers. However, using fluorescence detection in a conventional sequencer, these probes still give electropherograms which could easily be interpreted as shown in Figure 3 B-E. In Figure 3B and D, two examples of genomic DNA amplimers are shown, in Figure

3C and E the corresponding whole genome preamplified DNA samples are shown.

The fluorescence-based PCR method developed, is simple and rapid to perform and thus should be applicable to the identification of genetic polymorphisms within the IL-10 gene locus. This method provides the opportunity to analyse the microsatellite alleles of patients suffering from infectious, autoimmune or tumour disease to estimate their future risk for fatal outcome of the disease.

Investigation of IL-10IR, IL-10G and IL-10R microsatellites in Caucasian populations using the multiplex PCR

The established multiplex assay was used to analyse the allele frequencies of polymorphic IL-10 microsatellites in Caucasian and African donors. We compared the allele distribution in two independent donor groups from Germany comprising 112 (Northern Germany, Cologne) and 106 (Southern Germany, Stuttgart) donors respectively. The IL-10IR locus was nonpolymorphic in the analysed samples from Stuttgart (the cohort from Cologne was not analysed). The genetic differences for IL-10G and IL-10R between the donor groups from Cologne and Stuttgart are not statistically significant ($p > 0.05$) (Table 3). In total, we observed ten IL-10G and three IL-10R alleles in the Caucasian populations (Table 2). It was shown that the alleles IL-10G21 (37% in the Cologne population; 39% in the Stuttgart population) and G25 (21% in the Cologne population and 22% in the Stuttgart population) are the most common alleles in the groups studied in Germany. IL-10R13 was the allele with the highest frequency in Caucasians (69%). Assuming a Hardy-Weinberg distribution of genotypes, the degree of homo- and heterozygosity of Caucasian donors was estimated. Twenty-two percent of Caucasian donors are homozygous for IL-10G and 55% are homozygous for IL-10R. The most prevalent homozygous genotype found in the Caucasian population is IL-10G21/21, and genotypes IL-10G21/25 and G21/23 are the most common heterozygotes. In addition, most Caucasians are homozygous for IL-10R13/13 (50%) and heterozygous for IL-10R13/14 (36%). The genotypic distribution of both cohorts showed no significant deviation from the Hardy-Weinberg equilibrium, demonstrating that no significant genetic differences between either donor groups from Cologne and Stuttgart could be found.

Investigation of IL-10IR, IL-10G and IL-10R microsatellites in an African population using the multiplex PCR

The IL-10IR locus analysed spanning the inverted TG/CA repeat is nonpolymorphic in the analysed DNA from the African (Lambarene, Gabon, Central Africa) cohorts.

The analysis of 91 samples collected recently at the Albert-Schweitzer-Hospital in Lambarene, showed significant differences in the allele distribution for IL-10G and IL-10R compared to the Caucasian samples. This is shown by the genotypic variation for each population pair (Table 3). For both loci, IL-10G and IL-10R, the comparison between the results of the multiplex-PCR of the Stuttgart and Cologne groups *versus* the Lambarene group reveals highly significant differences ($p < 0.0001$) (Table 3). In Africans, the allele IL-10G25 is rare, at about 3% compared to 21% in Caucasians, alleles IL-10G22 and 23 are more prevalent in Africans as compared to Caucasians (22% *versus* 10% and 26% *versus* 9%, respectively) (Table 2). At the IL-10R microsatellite locus, the allele 12 is more often found in Africans (1% *versus* 0%), where-as alleles IL-10R14 and R15 are less frequent in the African group as compared to the Caucasian group with 9% *versus* 28% or 2% *versus* 3%, respectively (Table 2). Assuming a Hardy-Weinberg distribution of genotypes, the degree of homo- and heterozygosity of African donors was estimated (Table 4). In the African group analysed, the heterozygous genotype IL-10G21/25 is very rare at 1%, whereas in the Caucasian group, 13% are carrying both alleles. In the African group, the ratio of heterozygous to homozygous genotypes for the IL-10R locus is shifted towards the homozygous genotypes. This is due to the haplotype IL-10R13/13, which is more often found in the African samples than in the Caucasian samples (78% to 48%).

CONCLUSION

In the present report, a simple, two-colour fluorescence assay for the detection of microsatellite length by an automatic sequencer is described. Using this method, polymorphisms at the IL-10G and IL-10R loci of the 5' flanking region of the IL-10 gene were analysed simultaneously in a one tube PCR-reaction allowing a saving of time and DNA. The analysis of two different Caucasian populations using this multiplex PCR approach reveals no significant differences in allele frequencies between these donor groups, even though the blood was

collected in two cities in Germany, from different geographical regions North and South of the country. In contrast to this finding, the comparison of allele frequencies between these Caucasian populations and an African population from Gabon revealed significant differences for both microsatellites analysed. These genotypic patterns of the IL-10G and IL-10R microsatellites analysed demonstrate geographical polymorphism.

A unified nomenclature for the IL-10 microsatellite alleles is proposed, where the numbering directly reflects the number of CA repeats. Thus, allele IL-10G21 corresponds to the former IL-10G9, and IL-10R13 to the former IL-10R2 as described previously [27, 28, 38]. In the older nomenclature, the shortest IL-10G allele IL-10G1 has 12 dinucleotide repeats thus excluding all shorter alleles (even if they are very rare or indeed existing). The same is true for IL-10R, where the shortest IL-10R1 is characterized by 11 dinucleotide repeats. The proposed nomenclature is recommended since a growing number of publications about IL-10G and IL-10R has been observed, and the comparison of their data is very important for future clinical work.

These findings may form the basis for field studies about genetic predispositions in the development of infectious diseases, where IL-10 is deregulated. In malaria, we and others have found that deregulated IL-10 may correlate with defined subtypes of severe forms of disease or parasite clearance [22, 23, 39]. This forms the basis for analysis of the association of certain haplotypes of IL-10 with the expression of IL-10 in different clinical manifestations of malaria or other infectious diseases.

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Figures

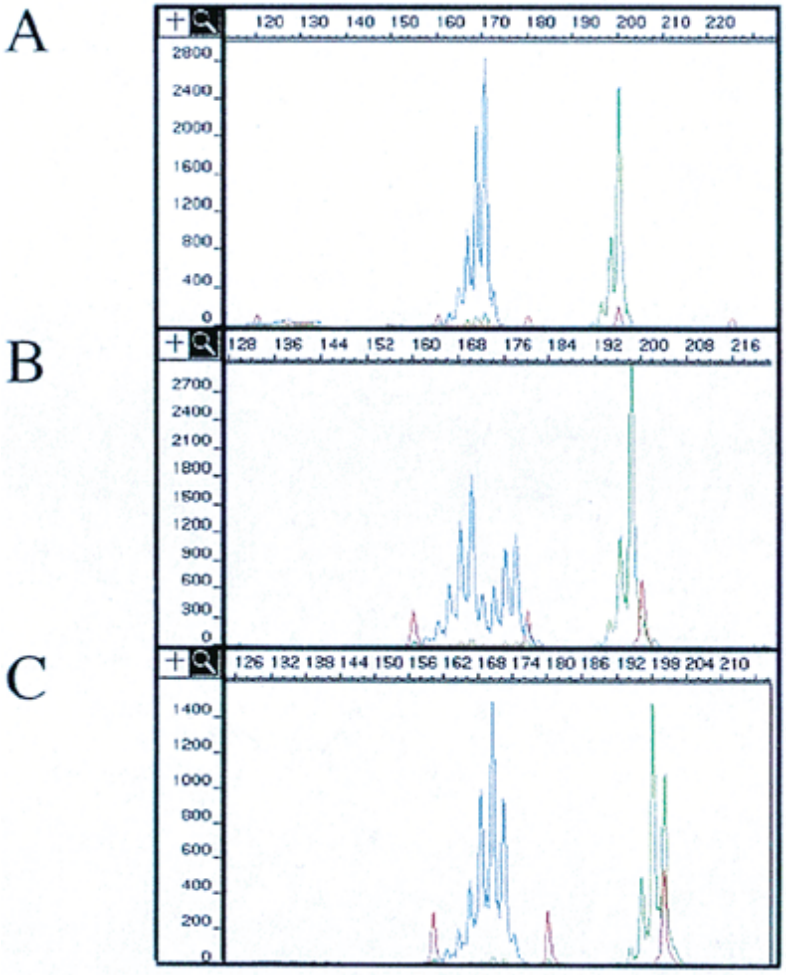


Figure 1
Representative examples of homozygous and heterozygous IL-10G and IL-10R alleles.

The electropherogram shows results of a multiplex PCR using primers IL-10G12-FAM/IL-10G23 and IL-10R11-HEX/IL-10R21 on 3 different genomic DNA samples. **A:** homozygous sample in IL-10G and IL-10R (G21; R14), **B:** heterozygous for IL-10G (4 CA repeats difference) and homozygous for IL-10R (G21/G25; R13), **C:** heterozygous for IL-10G (1 CA repeat difference) and heterozygous for

IL-10R (1 CA repeat) (G21/G22; R13/R14). Allele IL-10G21 corresponds to IL-10G9, and
IL-10R13 to IL-10R2 as described before [27, 28]. Allele sizes (in base pairs) are indicated on the top horizontal axis of each panel; relative fluorescence units are indicated on the vertical axis. Fluorescence-based PCR products were run on an ABI PRISM 377 autosequencer. PCR was performed with FAM (blue) and Hex (green) labeled primers. A red fluorescence-labeled marker (CXR) was included in each lane. One mug of genomic DNA and 100 pg of plasmid were amplified.

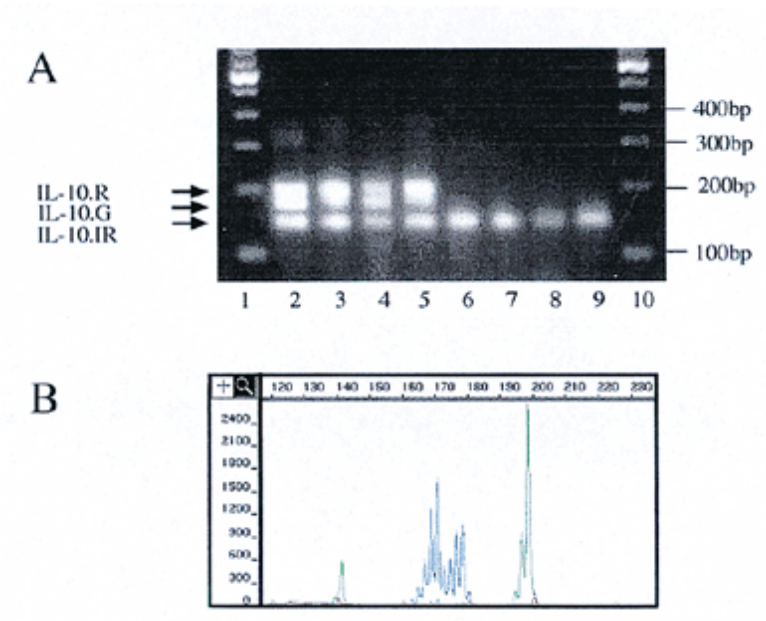


Figure 2
Introduction of a third primer pair into the two loci multiplex PCR.

A: example of an electropherogram of this multiplex PCR using primers IL-10TGCA-A/IL-10TGCA-B (for IL-10IR), IL-10G12-FAM/IL-10G23 (for IL-10G) and IL-10R11-HEX/IL-10R21 (for IL-10R) on genomic DNA (lanes 3-5 and 7-9). In lane 1 and 10, a 100 bp marker is shown. In lane 2 and 6, the plasmid amplimers are shown. One mug of genomic DNA

and 100 pg of plasmid were amplified, **B:** electropherogram of this multiplex PCR from the sample in lane 4 where IL-10IR is homozygous, IL-10G is heterozygous (G21/G25) and IL-10R is homozygous (R13).

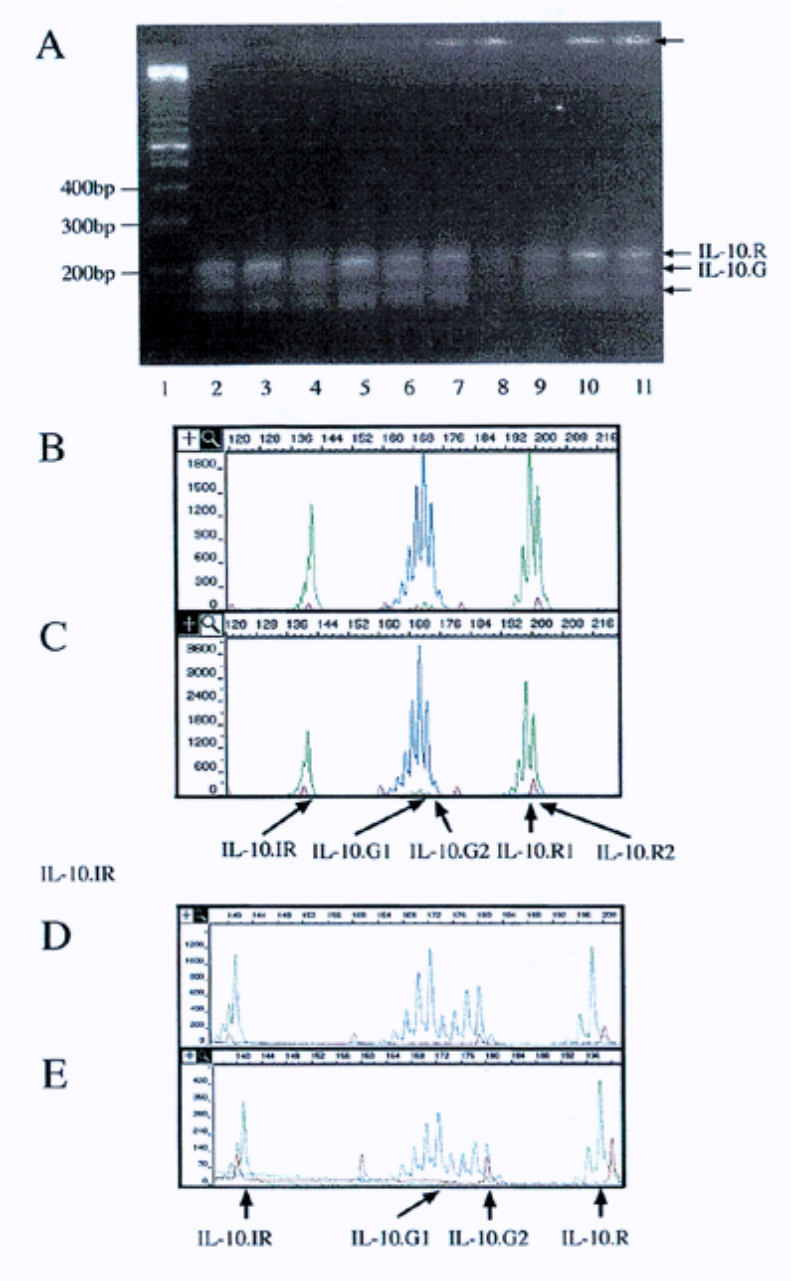


Figure 3
Whole genome multiplex - PCR with three pairs of primers.

A: agarose gel staining with ethidium bromide. Lanes 2-7, amplimers of a PCR from genomic DNA, and lane 8-11, amplimers from a PCR of corresponding preamplified DNA using primer IL-10TGCA-A/IL-10TGCA-B, IL-10G12-FAM/G23, IL-10R11-HEX/IL-10R21. In lane 1, a 100 bp marker is shown. **B** and **C**: electropherogram of PCR-fragments spanning the three IL-10 loci from donor 4 (lane 5/10) (homozygous for IL-10IR, heterozygous for IL-10G (G21, G22) and heterozygous for IL-10R (R13, R14)), and **D** and **E** from donor 5 (lane 6 /11) (homozygous for IL-10IR, heterozygous for IL-10G (G22, G26) and homozygous for IL-10R (R13). In **B** and **D** are the genomic DNA and in **C** and **E** the preamplified DNA are shown. The positions of IL-10IR, IL-10G1 and 2 and IL-10R1 and 2 are indicated by arrows in **C** and **E**.

Table 1
Oligonucleotides used

Name	Sequence
IL-10R11-HEX	5'-GCA CCC TCC AAA ATC TAT TTG CAT AAG-3'
IL-10R11-HEXa	5'-GTT TCT GTC CCT CCA AAA TCT ATT TGC ATA AG-3'
IL-10R21	5'-GTT TCT GTA GAG ATA GGA GGT GGT AAA GTC CA-3'
IL-10G12-FAM	5'-GTT TCT GTC AAC ACT CCT CGC CGC AAC CCA-3'
IL-10G22	5'-GTT TCT GTG GGG TGG AAG AAG TTG AAA TAA CAA GG-3'
IL-10G23	5'-GTT TCT GTG GTC CCT TAC TTT CCT CCT ACC TAT CC-3'
IL-10TGCA	5'-CTC ACT AAA GGG ATG GCT TTT ACA TAC CTC CCT-3'
IL-10TGCA-HEX	5'-TCA CTA TAG GGC GAG AGT GAC TCA GGG TG CAG-3'
MBL-f	5'-CAA AGG TAG GCA CTA TGA TGA GCA-3'
MBL-r	5'-CCA GAA AGT AGA GAG GTA TTT AGC-3'
ICAM1-f	5'-TCT TCC CTC GTT TCT TCT AGG ACC TGG-3'
ICAM-1r	5'-TCC GGG CTC AGT TAC TCA CAG TAC ACG-3'

Table 2
Allele frequencies of IL-10 microsatellites IL-10G and IL-10R in Caucasians (Germany) and Africans (Gabon) (in percent)

Allele		Caucasians n = 218	Africans n = 91
IL-10G			
	16	0	1
	17	0	0
	18	0	0
	19	3	4
	20	3	1
	21	38	29
	22	10	22
	23	9	26
	24	6	7
	25	21	3
	26	8	6
	27	1	1
	28	1	0
IL-10R			
	12	0	1
	13	69	88
	14	28	9
	15	3	2

Table 3
Genotypic differentiation for 3 populations from Stuttgart (South Germany), Cologne (North Germany) (Caucasians) and Lambarene (Gabon, Central Africa)(Africans). Analysis was performed using GENEPOP software [40]

Locus	Populations	p-value
IL-10G	Stuttgart-Cologne	0.911
IL-10G	Caucasians-Africans	< 0.0001
IL-10R	Stuttgart-Cologne	0.576
IL-10R	Caucasians-Africans	< 0.0001

Table 4
Expected and observed numbers of homo- and heterozygotes of IL-10G and IL-10R alleles in Caucasian (n = 218) and African (Lambarene n = 91) populations. Analysis was performed using GENEPOP software [40]

		Caucasian		African	
		homo-zygous	hetero-zygous	homo-zygous	hetero-zygous
IL-10G	Expected	51	181	19	72
	Observed	43	187	16	75
IL-10R	Expected	126	103	70	20
	Observed	131	97	72	18

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Plasmodium berghei, protection, rmGM-CSF, met-enkephalin.

Semiautomated and simultaneous analysis of the interleukin-10 gene microsatellites IL-10G and IL-10R by fluorescence-based polymerase chain reaction reveals significant differences in allele distributions between Caucasians (Germany) and Africans (Gabon)

D. Kube, D. Schmidt, M. Mörmann, A.C. Uhlemann, J. Tomiuk, H. Tesch, P.G. Kremsner
microsatellites, interleukin-10 promoter, multiplex-PCR, Caucasian, African.

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