Resistance to Collagen-induced Arthritis in a Nonhuman Primate Species Maps to the Major Histocompatibility Complex Class I Region

By Nicolaas P. M. Bakker,* Monique G. M. van Erck,* Nel Otting,* Neubury M. Lardy,‡ Riet C. Noort,* Bert A. 't Hart,* Margreet Jonker,* and Ronald E. Bontrop*

*From the *Institute for Applied Radiobiology and Immunology, 2280 HV Rijswijk; and the ‡Central Laboratory of the Red Cross Blood Transfusion Service, 1066 CX Amsterdam, The Netherlands*

Summary

Type II collagen–induced arthritis (CIA) is an experimentally inducible autoimmune disorder that is, just like several forms of human arthritis, influenced by a genetic background. Immunization of young rhesus monkeys (*Macaca mulatta*) with type II collagen (CII) induced CIA in about 70% of the animals. One major histocompatibility complex (MHC) class I allele was present only in young animals resistant to CIA and absent in arthritic animals. This strong association suggests that the MHC class I allele itself, or a closely linked gene, determines resistance to CIA. The mechanism controlling the resistance to CIA becomes less efficient in aged animals since older rhesus monkeys, which were positive for the resistance marker, developed a mild form of arthritis. At the cellular level it is demonstrated that resistance to CIA is reflected by a low responsiveness of T cells to CII. This association between a specified MHC class I allele and resistance to an autoimmune disease points at the importance of the MHC class I region in the regulation of the immune response to an autoantigen.

The highly polymorphic MHC region, which is known in humans as the human leukocyte antigen (HLA) system, plays a pivotal role in controlling the immune response to foreign pathogens. The MHC class I and II glycoproteins bind peptides derived from intracellular enzymatic degradation of proteins and subsequently present them on the cell surface to $CD8^+$ CTL and $CD4^+$ (primarily helper) T cells, respectively (1, 2). MHC class I and II region–encoded susceptibility genes for several autoimmune diseases have been documented in humans and experimental animals models (3–7). MHC genes associated with resistance to autoimmune disorders are relatively rare and have until now only been mapped to the class II region (8, 9).

Collagen-induced arthritis (CIA)[1] is an experimental animal model for studying immunoregulatory mechanisms underlying an autoimmune disease. Upon intradermal immunization with type II collagen (CII), the major cartilage protein, rhesus monkeys as well as rodents develop CIA that shares several pathologic features with human rheumatoid arthritis (RA) (10, 11). Human RA is associated with the HLA-DR1 and -DR4 serotypes (12, 13), while in mice susceptibility to CIA has been mapped to a critical site of an I-A allele encoded by the MHC class II region (14).

In this study the genetic influence of MHC genes on the development of CIA in rhesus monkeys was investigated. The current knowledge of the MHC of the rhesus monkey, recently named *MhcMamu* (15), located on chromosome 2, is primarily based on serological data. Allo-antisera allow the detection of 13 Mamu-A and 14 Mamu-B locus products while more than 10 allelic specificities are encoded by the Mamu-DR locus (16, 17). Recently, the knowledge of the MHC systems in different nonhuman primate species has expanded rapidly. These studies demonstrate that MHC polymorphisms are inherited in a trans-species mode of evolution (18, 19) which means that the evolution of MHC alleles does not start at the inception of a species, but is rather a process in which a major group of alleles is passed on in the phylogeny from one species to another. The major implication of this finding is that some alleles of different species are more related to each other than the MHC alleles within a species. This type of relationship may have functional implications as is reflected by antigen presentation studies across a species barrier (20). Therefore, the identification of MHC genes or alleles as markers for susceptibility or resistance to autoimmune dis-

[1] *Abbreviations used in this paper:* AS, ankylosing spondylitis; CIA, collagen-induced arthritis; CII, type II collagen; RA, rheumatoid arthritis.

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orders in nonhuman primate models may be relevant for understanding the pathogenesis and immunoregulation of human autoimmune diseases.

Materials and Methods

Animals. Most rhesus monkeys (*Macaca mulatta*) used in this study were born and raised at the ITRI-TNO Primate Center. Monkeys that were born outside the Primate Center are BB25 and BB37, born in the Zoo Beekse Bergen (Arnhem, The Netherlands); 3974 and 3982, born in Bethesda Primate Center (Bethesda, MD), and monkeys 2822, 2774, and 3215 were imported from India at a young age. Monkeys 4062, 4070, 4116, and 4106 were imported from Burma at a young age. All monkeys were typed for *MhcMamu* antigens using standard serological techniques (16). The animals used in the experiments were randomly selected from the colony. The sex and age of the animals are given in Table 1. The animals were examined, weighed, and bled under ketamine anesthesia. When an animal suffered from pain, 0.06 mg Buprenorfine was given twice daily by intramuscular injection (Temgesic, Warrick BV, The Netherlands). The experiments have been approved by the TNO ethical committee for animal experiments. Throughout the experiments the animals were under veterinary surveillance.

Collagens. Native bovine type II collagen (B-CII), isolated from bovine articular cartilage, was a kind gift from Dr. J. van Kampen, Dr. Jan van Breemen Institute (Amsterdam, The Netherlands). The isolation of B-CII and rhesus monkey CII (Rh-CII), respectively, from the bovine and rhesus monkey nasal septa has been described elsewhere (10).

Arthritis Induction. The animals were immunized by intracutaneous injection on the back with 1 ml of an emulsion (1:1) of purified B-CII or Rh-CII dissolved in 0.1 M acetic acid and CFA (Difco Laboratories, Detroit, MI). CII doses in the primary immunizations are given in Table 1. The disease severity was quantified in an arthritic score as explained in the legend to Table 1.

Proliferation of Mononuclear Cells. PBMC were isolated from heparinized blood by density gradient centrifugation (LSM; Organon Technika, Durham, NC). The cells from the interphase of the gradient were washed twice with HBSS (Gibco Ltd., Paisley, Scotland) and resuspended in culture medium, consisting of Hepes-buffered RPMI 1640 (Gibco Ltd.) supplemented with L-glutamine (200 mM; Gibco Ltd.), 0.15% $NaHCO_3$, 200 U/ml penicillin, 200 U/ml streptomycin (both Gibco Ltd.), and 20% heat-inactivated pooled nonimmune rhesus monkey serum. PBMC were cultured for 6 d in 96-well round-bottomed plates (Greiner, Labortechnik, Germany) in a humidified atmosphere containing 5% CO_2 at 37°C at a concentration of 5×10^4 cells per well. Denatured CII was added in the optimal concentration of 50 μg/ml. Cell proliferation was measured by the incorporation of [^{3}H]thymidine (0.5 μCi per well) during the final 8 h of the culture.

Results and Discussion

After immunization of 24 MHC-typed young animals (<6.5 yr of age) with heterologous or homologous CII, 16 young animals developed CIA (Table 1). No apparent association was observed between susceptibility, sex, and immunization dose of CII or between Mamu-DR and CIA. However, seven of eight resistant animals shared the same serologically defined MHC class I allele (Mamu-A26), which was absent in all 18 young animals that developed CIA ($p < 0.00002$). Only one of the resistant animals (8675) lacks the Mamu-A26 allele (Table 1). To our knowledge this is the first demonstration that resistance to an autoimmune disease, which can be evoked by immunization with a soluble antigen, is associated with the MHC class I region. So far, resistance markers for autoimmune diseases have been exclusively linked to the MHC class II region (8, 9). The Mamu-A26-associated resistance seems unique for CIA since this phenomenon was not observed in rhesus monkeys immunized with myelin basic protein to evoke experimental autoimmune encephalomyelitis (data not shown). Because in both models immunization was performed with CFA, it is unlikely that the Mamu-A26-associated resistance is due to the adjuvant.

It is generally accepted that during aging the capacity of the immune system to maintain tolerance to autoantigens decreases. To study whether the association between Mamu-A26 and resistance to CIA holds true in old animals, one male and four female monkeys of >22 yr of age were immunized with CII. The Mamu-A26-positive old animals (3215 and 2822) developed arthritis, but the severity of the disease was clearly less than in the Mamu-A26-negative animals (Table 1).

Using biochemical methods the MHC class I gene products were characterized in more detail. One-dimensional IEF of class I products, immunoprecipitated by using the anti-MHC class I mAb W6/32, demonstrated that the Mamu-A and -B serotypes correlate with isoelectric point differences (manuscript in preparation). In addition, such experiments also showed that all Mamu-A26-positive animals share one product with a similar isoelectric point that was absent in the Mamu-A26-negative animals.

There appears to exist a strong correlation between the Mamu-A26 allele and resistance to develop CIA. The question arises, whether Mamu-A26 itself confers resistance, or whether it functions as a marker for a closely linked regulator gene. At present it is not known whether the Mamu-A alleles are equivalent to the HLA-A or -B alleles. Therefore, we can not exclude that other genes, like some of the HLA-B-associated transcripts (BAT), exhibit polymorphism and may be linked to Mamu-A26 (21–23). In this respect, it is of interest that some BAT genes encode for collagen-like structures (24). A potential problem with any association study is population stratification. In this case, the A26 allele may mark a subset of rhesus monkeys resistant to CIA because of other genes in that subset. In this respect, we are studying V_β usage in TCR diversity in order to elucidate whether other polymorphic gene systems may play a role in resistance to CIA. The alternative option is that the Mamu-A26 allele determines resistance by itself. MHC class I molecules present peptides from intracellular origin to CTL (1), whereas soluble antigens derived from extracellular pathogens are usually presented by MHC class II molecules (2). When the Mamu-A26 allele itself is involved in resistance to CIA, the question has to be answered if Mamu-A26 molecules are able to bind self-peptides from CII, which is a soluble antigen. This is not unlikely because it has been documented recently that peptides derived from soluble proteins can complex with class

Table 1. *Resistance to Collagen-induced Arthritis in Rhesus Monkeys is Associated with Mamu-A26*

Animals	Rhesus monkey	Sex	Age‡	*MhcMamu** A	*MhcMamu** B	*MhcMamu** DR	Immunization dose§	Anti-CII T cell response	Arthritis¶
			yr				*mg*		
Young	BB25	♂	6.0	–,–	10,–	3,8	1.5	+	+ + +
	2BN	♂	3.5	11,18	9,19	3,4	1.5	+	+ + +
	BB37	♀	3.0	–,31	–,–	3,2	1.5	+	+ + +
	1WF	♀	4.0	11,32	9,28	2,5	1.5	+	+ + +
	1JT	♀	6.5	11,24	19,33	3,2	1.5	+	+ + +
	1KM	♀	6.0	11,14	1,19	3,1	3.0	+	+ +
	D3	♂	4.0	17,18	3,28	1,1	1.5	+	+ +
	M15	♂	4.0	11,2	6,21	3,8	3.0	+	+ +
	1OX	♀	5.0	11,13	9,9	–,8	3.0	+	+ +
	L65	♀	5.0	–,2	–,–	2,8	1.5	+	+ +
	4062	♂	6.0	11,20	10,–	2,3	1.0	+	+ +
	4116	♂	7.0	18,29	33,–	2,4	1.0	+	+ +
	4070	♂	8.0	2,31	6,–	2,–	1.0	+	+
	8827	♂	1.5	2,11	10,27	1,2	1.0	+	+ (s)
	8765	♀	3.0	17,32	10,27	101,8	1.0(r)	+	+ (s)
	8684	♀	4.0	2,17	10,22	8,1	1.0(r)	–	+ (s)
	8675	♂	3.0	2,11	6,10	1,3	1.0	–	–
	M14	♂	4.0	**11,26**	6,19	2,8	3.0	–	–
	1UY	♂	3.5	**11,26**	19,27	1,2	3.0	–	–
	1RO	♀	4.5	**24,26**	6,–	3,3	1.0	–	–
	1NR	♂	5.5	**11,26**	21,23	5,101	1.0	–	–
	8769	♂	2.0	**11,26**	27,19	1,2	1.0	+	–
	8781	♀	3.0	**2,26**	19,23	3,3	1.0(r)	–	–
	4106	♀	6.0	**–,26**	6,–	8,5	1.0(r)	–	–
Old	2774	♂	>23	11,32	10,19	3,101	1.0	+	+ +
	3974	♀	>23	2,13	6,10	4,1	1.0	+	+ + +
	3982	♀	>22	14,32	6,23	4,5	1.0	+	+ + +
	3215	♀	>23	**13,26**	9,6	5,3	1.0	+	+ (s)
	2822	♀	>22	**31,26**	33,6	1,3	1.0	+	+

* *MhcMamu*-A, -B, and -DR locus alleles have been identified serologically (16). The A26 allele has been printed in bold.
‡ Age at time of immunization. In captivity rhesus monkeys can reach a maximum of 30–35 yr of age.
§ All rhesus monkeys are immunized with B-CII. (r) Animals immunized with Rh-CII.
‖ The CII-dependent T cell proliferation is scored as negative (–) if the stimulation indices (cpm from cells cultured with CII/cpm from cells cultured only in culture medium) was <2 during the first 25 wk after the primary immunization with CII. A positive score (+) is given when the stimulation indices are >2. The anti-CII T cell response in the responding animals ranged from 4,000 to 23,000 cpm, whereas the nonresponder animal reached levels of 300 cpm, which is approximately equal to the controls, done with medium with no antigen.
¶ The arthritic score is as follows: + + +, severe soft tissue swelling of all small joints of hands and feet and of the wrists and ankle joints; + +, moderate soft tissue swelling of most small joints of hands and feets and wrists and ankle joints; +, moderate soft tissue swelling only of wrists and ankle joints; + (s), sub-clinical arthritis only manifest by elevated erythrocyte sedimentation rate and C-reactive protein levels and the detection of anti-CII T cell response and IgM antibodies (30).

I molecules (25). As shown in Table 1, resistance to CIA in rhesus monkeys is accompanied by in vitro CII-specific T cell low responsiveness. The binding of peptides from CII by Mamu-A26 molecules and subsequent presentation to regulator CD8-positive T cells, which can eliminate APC such as CII-reactive B cells and cells of the macrophage lineage (26), may be an effective mechanism in the prevention of CIA. After repeated immunizations with CII, all arthritic monkeys

acquire resistance to the disease, which is also reflected by a T cell low responsiveness to CII. Therefore, it is possible that other class I alleles, although less efficiently than A26, mediate resistance to CIA in a similar manner.

Findings in the human population on associations between certain HLA class I alleles and protection to disease are mainly observed for infectious diseases (27, 28). On the other hand, there exists a strong counterpart of the MHC class I–linked resistance to CIA in nonhuman primates, namely the association between HLA-B27 and ankylosing spondylitis (AS) (3, 4), which is an inflammatory disease predominantly affecting the joints of the spine and the pelvis. Although MHC class I determines in AS susceptibility and in CIA resistance to disease, both associations are remarkably strong. At present it is disputed whether AS is an autoimmune or an infectious disease (29). Apart from that, the antigen that causes AS has not been identified. However, in the case of CIA, disease is evoked by a known autoantigen, namely CII. Therefore, this arthritis model is most suitable to investigate the role of MHC class I genes in the immunoregulation of autoimmune arthritis in primates.

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Address correspondence to Dr. N.P.M. Bakker, Institute for Applied Radiobiology and Immunology TNO, P.O. Box 5815, 2280 HV Rijswijk, The Netherlands.

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