Major Histocompatibility Complex in the Rat and Blood Pressure Regulation

Jaroslav Kuneš, Milada Kohoutová, and Josef Zicha

The objective of this study was to evaluate whether the major histocompatibility complex of the rat can be related to blood pressure (BP) level and BP response to stress. Blood pressure was determined under light ether anesthesia or during moderate restraint stress in normotensive Lewis rats, in rat strains congenic with respect to their RT1 haplotype [LEW.1A (RT1^a) and LEW.1W (RT1^u)], and in their recombinant lines LEW.1AR1 (RT1^{ar1}), LEW.1AR2 (RT1^{ar2}), LEW.1WR1 (RT1^{wr1}), and LEW.1WR2 (RT1^{wr2}). Under light ether anesthesia, systolic blood pressure was similar in Lewis, LEW.1A, and LEW.1W rats. There were also no significant differences in blood pressure in LEW.1AR1 and LEW.1AR2 animals when compared with Lewis or LEW.1A rats. In contrast, BP was significantly increased in LEW.1WR2 rats. On the other hand, moderate restraint stress induced a BP increase in animals of all recombinant lines compared to the respective congenic strains.

These results confirmed our previous finding in recombinant inbred strains about the significant role of RT1 complex in BP regulation. Moreover, our data indicated that BP can be influenced by interaction of individual regions of the RT1 complex on the genetic background of Lewis strain. Am J Hypertens 1996;9:675–680

KEY WORDS: **Hypertension, immune system, RT1 complex, stress, congenic strains, major histocompatibility complex, Lewis rat.**

There is increasing evidence that genetic factors may also act through immunologic pathways in the pathogenesis of hypertension.[1] Several studies used human leukocyte antigens (HLA) as markers for human essential hypertension.[2–6] The significant link between essential hypertension with HLA DR2[7], DR4[8], and DR7[6] was reported in several studies. Gerbase-DeLima et al.[7] found a high frequency of the haplotype HLA-A2B12 as well as DR2 and DR4 in hypertensive siblings. Nevertheless, the role of HLA antigen in patients with essential hypertension remains controversial. This may be explained by the variable role of the HLA antigen system in the etiology of essential hypertension in different ethnic groups.

Studies comparing the frequency of HLA antigen in different patient populations with the frequency present in control populations have shown that a high percentage of autoimmune diseases is influenced by the major histocompatibility complex (MHC).[9] The concept of hypertension as an autoimmune disease has also been proposed.[1] A direct involvement of either the products of the polymorphic class I and class II or the less variable products of the class III genes has been discussed.[10] It has become evident that genes in the class III region could be of interest. This region contains a heterogeneous group of genes encoding

Received November 30, 1994. Accepted December 11, 1995.

From the Institute of Physiology (JK, JZ), Academy of Sciences of the Czech Republic and Institute of Biology (MK), First Faculty of Medicine, Charles University, Prague, Czech Republic.

This study was partially supported by research grants 711104 (Academy of Sciences of the Czech Republic) and 306/93/0573 (Grant Agency of the Czech Republic) within the frame of EURHYPGEN Concerted Action of the European Community.

Address correspondence and reprint requests to Dr Jaroslav Kuneš, Institute of Physiology, Vídeňská 1083, CZ-142 20 Prague 4, Czech Republic.

0895-7061/96/$15.00
PII S0895-7061(96)00023-4

three components of the complement system (ie, C2, factor B, and C4)[11,12] the microsomal cytochrome P-450 steroid 21-hydroxylase,[13] the cytokines tumor necrosis factor-α and -β,[14,15] and others. Recently, the gene for major heat shock protein (*hsp70*) was recognized within MHC.[16,17]

The rat MHC (RT1 complex) is located on chromosome 20[18] and encompasses a chromosomal region of about 2 to 4 cM according to recombination data. The Glo-1r and Acry-1 genes are located in the neighborhood of the RT1 complex.[19,20] These genes affect the enzyme glyoxalase-1 and A-chain of α-crystallin, respectively. The given haplotypes of RT1 complex are associated with susceptibility or resistance to certain diseases (eg, insulin-dependent diabetes mellitus).[21]

It has been demonstrated that the immune system of spontaneously hypertensive rats (SHR) is depressed and the chronic phase of hypertension is thymus-dependent.[22] Not only were fewer T lymphocytes reported in adult SHR rats but there was even a significant decline of this cell population during aging in contrast to Wistar-Kyoto rats in which the number of T lymphocytes increased with age. It was also noted that the functional capability of T lymphocytes in SHR was depressed. Moreover, manipulation of the immune system with immunosuppressive treatment attenuated hypertension development in SHR.[23] Recently, immune system abnormalities were disclosed even in 2-week-old SHR rats, favoring the hypothesis that the immunologic disturbances are of a primary nature.[24]

The immune system is also implicated in the cause of hypertension in other animal models of spontaneous hypertension. Neonatal removal of thymus attenuated hypertension development in Lyon hypertensive rats.[25] New Zealand Black (NZB) mice developed spontaneous hypertension,[26] but their substrain, which is genetically athymic (nude NZB), remained normotensive. Immunosuppressive therapy with cyclophosphamide normalized blood pressure in NZB mice. Moreover, grafting of thymus tissue from NZB mice into nude NZB ones induced hypertension.[26]

It was demonstrated by use of recombinant inbred (RI) strains that genes within the RT1 complex or closely linked to it are associated with blood pressure.[27] In addition, we have found that the polymorphism of the *hsp70* gene between normotensive and hypertensive rats was associated with a blood pressure difference of 15 mm Hg in RI strains.[28] This was not revealed by Lodwick et al,[29] who found no evidence of linkage between blood pressure and the *hsp70* gene locus or other genes located within the RT1 complex. The reasons for the discrepant findings are unclear, but they could reflect the use of different control strains or different techniques of blood pressure monitoring.

The aim of this study was to evaluate further the involvement of the genes of the rat's major histocompatibility complex (RT1 complex) in relation to blood pressure levels and blood pressure response to stress. The combination of inbred strain with its congenic partners and with its recombinant lines is an ideal tool for studying the influence of one particular chromosomal segment on the same genetic background.

METHODS

We used adult (6-month-old) male Lewis normotensive rats (LEW, *l* haplotype of RT1 complex) as well as inbred congenic strains LEW.1A (RT1^{a}) and LEW.1W (RT1^{u}) in which the RT1 complex of AVN (*a* haplotype) and Wistar Prague (*u* haplotype) was transferred to Lewis genetic background by repeated backcrossing.[30] In addition, recombinant lines LEW.1AR1 (RT1^{ar1}, r2), LEW.1AR2 (RT1^{ar2}, r3), LEW.1WR1 (RT1^{wr1}, r4), and LEW.1WR2 (RT1^{wr2}, r6)[31–33] were used in this study. The recombinant haplotypes of the RT1 complex were originally detected during combined serologic and histogenetic screening program of segregating hybrids derived from RT1 congenic parental strains LEW.1A and LEW.1W.[31–33] All six inbred strains were bred by brother–sister mating in Prague and were routinely checked for isohistogeneity by intrafamiliar skin graft exchange. They represent the full haplotypes RT1^{a} and RT1^{u} and recombinant haplotypes between them enabling us to distinguish particular regions of the RT1 complex (Table 1) (for review see ref. 34). A molecular genetic approach made possible a more precise description of regions in the rat RT1 complex. The RT1.A region codes the classic class I antigens, the RT1.B/D region determines the class II antigens, and the RT1.C region encodes the class I-like antigens.[35]

All animals were maintained in air-conditioned facilities, at constant temperature (23°C), with a 12-h light-dark cycle, on a normal rat chow and with free access to tap water.

Blood pressure of conscious animals was measured by tail-cuff plethysmography immediately after placing the animals into a holder without previous training. The mean of five readings was taken as final blood pressure value, which corresponds to moderate restraint stress. The following day, systolic, mean arterial, and diastolic blood pressures were measured under light ether anesthesia using P23 Db Statham transducer (Valley View, OH) and Hewlett-Packard recorder (Andover, MA). Animals were killed and the weights of the hearts and kidneys were determined.

All data were expressed as means ± SEM. Statistical analysis was done using Student's *t* test or one-way analysis of variance followed by the calculation of

TABLE 1. RT1 COMPLEX AND RECOMBINANT SITES OF VARIOUS RT1 HAPLOTYPES

Strain	Haplotype	Class I RT1.A	Class II RT1.B/D	Class III C4	Class III Bf	Class III Hsp	Class I-like RT1.C
LEW	l	l	l	l	l	l	l
LEW.1A	a	a	a	a	a	a	a
LEW.1W	u	u	u	u	u	u	u
LEW.1AR1	r2	a |	u	u	u	u	u
LEW.1AR2	r3	a	a |	u	u	u	u
LEW.1WR1	r4	u	u	u	u |	a	a
LEW.1WR2	r6	u |	a	a	a	a	a

Data are derived from refs. 12, 17, and 35. The vertical bars indicate the recombinant sites.

least significant differences.[36] Values of $P < .05$ were considered as significant.

RESULTS

Table 2 summarizes the body weight, relative organ weights, as well as systolic blood pressure during moderate restraint stress in all strains studied. Body weight was comparable in all strains except the congenic strain LEW.1W and the recombinant line LEW.1WR1. During restraint, stress systolic blood pressure of the congenic strain LEW.1W was significantly lower in comparison with the Lewis strain. Systolic blood pressure of all recombinant lines was elevated compared to the respective congenic strains, three of them having even significantly higher blood pressure than Lewis rats (Table 2). Both relative heart and kidney weights were significantly lower in almost all strains when compared to Lewis animals. The only exception was recombinant line LEW.1AR2, which did not differ from Lewis rats.

Systolic blood pressure measured under light ether anesthesia was similar in Lewis and both congenic strains (Figure 1). The only blood pressure elevation was observed in recombinant line LEW.1WR2 in comparison with either Lewis, LEW.1W, or LEW.1AR2 rats (Figure 1). The same was true for diastolic blood pressure.

DISCUSSION

Results of this study confirmed our previous suggestion that the genes of MHC of the rat (RT1 complex) might be involved in blood pressure regulation.[27] Moreover, the involvement of the genes within the RT1 complex may be relevant for the environmental response implicated in the development of hypertension.[28,37] As discussed in the introduction, genes predominantly in the class III region could be involved in this process, but there is still high probability that the genes of class I or II are also involved. However, because of the linkage disequilibrium displayed by alleles within the MHC, it is not clear in many cases whether the increased susceptibility is due to the products of particular class I, II, or III loci per se, due to a combination of alleles at various loci, or due to the products of genes not yet identified.

Recently we have shown the association between RT1 complex and relative heart weight.[38] Our present study demonstrates the different impact of individual RT1 haplotypes on both relative heart and kidney weight. Because of the same genetic background it

TABLE 2. BODY WEIGHT (BW), RELATIVE HEART (HW/BW), AND RELATIVE KIDNEY (KW/BW) WEIGHTS AS WELL AS INDIRECT SYSTOLIC BLOOD PRESSURE (SBP_{IN}) MEASURED DURING MODERATE RESTRAINT STRESS IN ALL STRAINS STUDIED

Strain (n)	BW (g)	HW/BW (mg/100 mg BW)	KW/BW (mg/100 mg BW)	SBP_{in} (mm Hg)
Lewis (n = 12)	330 ± 7	242 ± 2	650 ± 1	112 ± 3
LEW.1A (n = 9)	349 ± 9	218 ± 4*	579 ± 8*	110 ± 2
LEW.1AR1 (n = 11)	322 ± 9	217 ± 5*	575 ± 12*	124 ± 1*†
LEW.1AR2 (n = 11)	340 ± 5	233 ± 5‡	637 ± 13†	137 ± 1*†
LEW.1W (n = 10)	360 ± 4*	218 ± 2*	624 ± 5*	92 ± 1*
LEW.1WR1 (n = 11)	372 ± 8*	212 ± 2*	610 ± 7*	111 ± 1†
LEW.1WR2 (n = 11)	340 ± 5	228 ± 3*‡	608 ± 14*	131 ± 1*†

** $P < .01$ significantly different from Lewis rats; † $P < .01$, ‡ $P < .05$ significantly different from the respective congenic strain (LEW.1A or LEW.1W).*

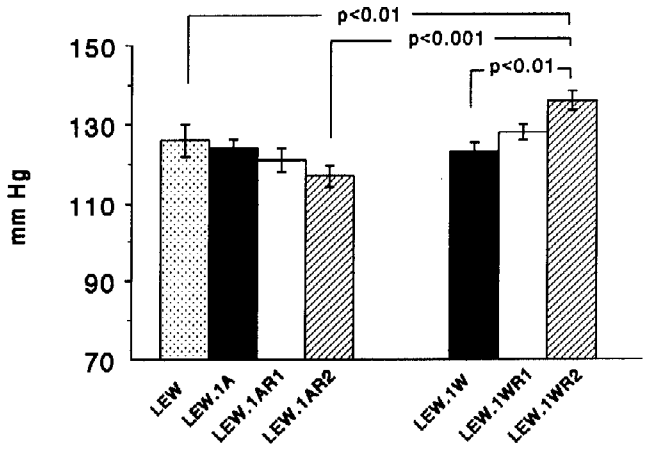

FIGURE 1. *Systolic blood pressure measured under light ether anesthesia.*

could be speculated that the genes within *a* and *u* haplotypes can decrease relative heart and kidney weight in comparison with the *l* haplotype. The use of recombinant lines further revealed that the gene-to-gene interaction inside the RT1 complex might be very important because the second recombination increased relative heart weight in both recombinant lines (LEW.1AR2 and LEW.1WR2), whereas relative kidney weight was increased only in LEW.1AR2. The candidate genes involved in heart and kidney weight determination might be searched for predominantly within the growth and reproduction complex that is located in the RT1 complex.[39]

There are several known genes in the RT1 complex with potential cardiovascular implications (including those for tumor necrosis factor-α, 21-hydroxylase, and *hsp70*), but there could be many as yet not identified genes. The impact of the genes of the RT1 complex on blood pressure was recognized in the set of recombinant inbred (RI) strains.[27] In the same set of RI strains we have found that the *hsp70* restriction fragment length polymorphism was associated with a blood pressure difference of 15 mm Hg.[28] This was not revealed in the study by Lodwick et al.[29] in SHR × Wistar-Kyoto crosses who found no linkage between the *hsp70* gene locus or other genes located within the RT1 complex and blood pressure. These researchers discussed several explanations for these discrepant results. One of them was that the genetic background provided by Lodwick's cross may have prevented the expression of the effect of the SHR-specific locus (in this case *hsp70* locus) on blood pressure. The results of our study support this explanation. The use of congenic strains with their recombinant lines suggested that the gene-to-gene interaction can play a significant role in the expression of genes. It was evident that even on "normotensive" genetic background the interaction of certain gene regions within the RT1 complex (recombinant LEW.1WR2 line) led to blood pressure increase not only during moderate restraint stress but even under light ether anesthesia. Recently we have found that genes within or close to the RT1 complex are partially responsible for the salt sensitivity of the rat.[40] The more detailed genetic analysis of the RT1 complex including its interaction with other genes throughout the genome would be necessary to clarify its role in the control of blood pressure level.

In conclusion, our results confirmed our previous observations about the significant role of the RT1 complex in blood pressure regulation. Moreover, it is evident that at least in some genetic "combinations" the genes of the MHC might be implicated in hypertension development per se or in higher susceptibility to environmental stimuli. The further analysis of these mechanisms might be rather difficult due to the genetic complexity that includes multiple alleles, differences in genetic background, linkage relationships, and genotype-environment interaction.[41] The net effect of these factors makes many results strain- and cross-specific. Therefore, it would be naive to expect comparable blood pressure effects of particular loci under various experimental conditions.

REFERENCES

1. Dzielak DJ: The immune system and hypertension. Hypertension 1992;19(suppl I):I36–I44.
2. Gelshorpe K, Doughty RW, Bing RF, et al: HLA antigens in essential hypertension. Lancet 1975;i:1039–1040.
3. Gudbrandsson P, Herlitz H, Hansson L, Rydberg L: Human leukocyte antigens in patients with previous essential malignant hypertension. Clin Sci 1980; 59:431s–434s.

4. Patel R, Johnson J: Histocompatibility antigens in black patients with essential hypertension. Circulation 1981; 64:1042–1044.
5. Shkhvatsabaya IK, Osipov SG, Suvorov I, et al: HLA antigens and the complement system in essential hypertension. Cor Vasa 1984;26:408–414.
6. Sengar DPS, Conture RA, Jindal SL, Catching JD: Histocompatibility antigens in essential hypertension and myocardial infarction. Tissue Antigens 1985;26:168–171.
7. Gerbase-DeLima M, DeLima JJG, Persoli LB, et al: Essential hypertension and histocompatibility antigens. A linkage study. Hypertension 1989;14:604–609.
8. Gerbase-DeLima M, Ladalardo MA, DeLima JJG, et al: Essential hypertension and histocompatibility antigens. An association study. Hypertension 1992;19:400–402.
9. Batchelor JR, McMichael AJ: Progress in understanding HLA and disease associations. Br Med Bull 1987; 43:156–183.
10. Todd JA, Acha-Orbea H, Bell JI, et al: A molecular basis for MHC class II-associated autoimmunity. Science 1988;240:1003–1009.
11. Carroll MC, Campbell RD, Bentley DR, Porter RR: A molecular map of the human major histocompatibility complex class III region linking complement genes C4, C2 and factor B. Nature 1984;307:237–241.
12. Wurst W, Rothermel E, Günther E: Genetic mapping of C4 and Bf complement and 21-hydroxylase genes in the rat major histocompatibility complex. Immunogenetics 1988;28:57–60.
13. Carroll MC, Campbell RD, Porter RR: Mapping of steroid 21-hydroxylase genes adjacent to complement component C4 genes in HLA, the major histocompatibility complex in man. Proc Natl Acad Sci USA 1985; 82:521–525.
14. Carroll MC, Katzman P, Alicot EM, et al: Linkage map of the human major histocompatibility complex including the tumor necrosis factor genes. Proc Natl Acad Sci USA 1987;84:8535–8539.
15. Dunham I, Sargent CA, Trowsdale J, Campbell RD: Molecular mapping of the human major histocompatibility complex by pulsed-field gel electrophoresis. Proc Natl Acad Sci USA 1987;84:7237–7241.
16. Sargent CA, Dunham I, Trowsdale J, Campbell RD: Human major histocompatibility complex contains genes for the major heat shock protein HSP 70. Proc Natl Acad Sci USA 1989;86:1968–1972.
17. Wurst W, Benesch C, Drabent B, et al: Localization of heat shock protein 70 genes inside the rat major histocompatibility complex close to class III genes. Immunogenetics 1989;30:46–49.
18. Levan G, Hanson C, Klinga K, Szpirer C: The rat gene map 1989. Rat News Lett 1990;23:12–14.
19. Stolc VH, Kunz HW, Gill TJ, III: The linkage of glyoxalase-I to the major histocompatibility complex in the rat. J Immunol 1980;125:1167–1170.
20. Skow LC, Kunz HW, Gill TJ, III: Linkage of the locus encoding the A-chain of alpha-crystallin (Acry-1) to the major histocompatibility complex in the rat. Immunogenetics 1985;22:291–293.
21. Günther E, Kiesel U, Kolb H, et al: Genetic analysis of susceptibility to diabetes mellitus in F2-hybrids between diabetes-prone BB and various MHC-recombinant congenic rat strains. J Autoimmun 1991;4:543–551.
22. Takeichi N, Suzuki K, Okayasu T, Kobayashi H: Immunological depression in spontaneously hypertensive rats. Clin Exp Immunol 1980;40:120–126.
23. Khraibi AA, Norman RA Jr, Dzielak DJ: Chronic immunosuppression attenuates hypertension in Okamoto spontaneously hypertensive rats. Am J Physiol 1984; 247:H722–H726.
24. Fannon LD, Braylan RC, Phillips MI: Alterations of lymphocytes populations during development in spontaneously hypertensive rat. J Hypertens 1992;10:629–634.
25. Bataillard A, Freiche JC, Vincent M, et al: Effects of neonatal thymectomy on blood pressure and immunological characteristics of genetically hypertensive rats of Lyon strain. J Hypertens 1986;4:545–547.
26. Svendsen UG: Spontaneous hypertension and hypertensive vascular desease in the NZD strain of mice. Acta Pathol Microbiol Scand 1977;85:548–554.
27. Pravenec M, Klír P, Křen V, et al: An analysis of spontaneous hypertension in spontaneously hypertensive rats by means of new recombinant inbred strains. J Hypertens 1989;7:217–222.
28. Hamet P, Kong D, Pravenec M, et al: Restriction fragment length polymorphism of *hsp70* gene, localized in the RT1 complex, is associated with hypertension in spontaneously hypertensive rats. Hypertension 1992; 19:611–614.
29. Lodwick D, Kaiser MA, Harris J, et al: Failure of the heat-shock protein 70 locus to cosegregate with blood pressure in spontaneously hypertensive rat × Wistar-Kyoto rat cross. J Hypertens 1993;11:1047–1051.
30. Štark O, Křen V: Five congenic resistant lines of rats differing at the rat H-1 locus. Transplantation 1969; 8:200–203.
31. Štark O, Günther E, Kohoutová M, et al: Genetic recombination in the major histocompatibility complex (H-1, Ag-B) of the rat. Immunogenetics 1977;5:183–187.
32. Kohoutová M, Günther E, Štark O: Genetic definition of a further gene region and identification at least three different histocompatibility loci in the RT1 complex. Immunogenetics 1980;11:483–490.
33. Kohoutová M, Štark O, Kormúth E, Günther E: The recombinant RT1 r6 haplotype of the LEW.1WR2 strains. Transplant Proc 1981;13:1322–1324.
34. Hedrich HJ: List of congenic and segregating inbred strains, *in* Hedrich HJ (ed): Genetic Monitoring of Inbred Strains of Rats. Stuttgart, Gustav Fischer Verlag, 1990, pp 481–486.
35. Günther E, Wurst W, Wonigeit K, Epplen JT: Analysis of the rat major histocompatibility system by Southern blot hybridization. J Immunol 1985;134:1257–1261.

36. Snedecor GW, Cochran WG: Statistical Methods. Ames, Iowa State University Press, 1968, pp 258–298.

37. Pravenec M, Sun Y2DL, Kuneš J, et al: Environmental susceptibility in hypertension: potential role of *HSP70* and *TNFα* genes. J Vasc Med Biol 1991;3:297–302.

38. Kuneš J, Křen V, Klír P, et al: Genetic determination of heart and kidney weights studied using a set of recombinant inbred strains: the relationship to blood pressure. J Hypertens 1990;8:1091–1095.

39. Kunz HW, Gill TJ, Dixon PD, et al: Growth and reproduction complex in the rat: genes linked to the MHC that affect development. J Exp Med 1980;152:1506–1518.

40. Kuneš J, Zicha J: Association of salt sensitivity in the rat with genes of major histocompatibility complex. Hypertension 1994;24:645–647.

41. Rapp JP, Dene H, Deng AY: Seven renin alleles in rats and their effects on blood pressure. J Hypertens 1994; 12:349–355.