—Note—

Antinucleolar Autoantibody Induced in Mice by Mercuric Chloride —Strain Difference in Susceptibility—

Junzo SAEGUSA, Yoshihiro KIUCHI*, and Toshio ITOH**

*National Institute of Industrial Health, 6-21-1 Nagao, Tama-ku, Kawasaki-shi, Kanagawa 214, *School of Medicine, Yokohama City University, 3-9 Fukuura, Kanazawa-ku, Yokohama-shi, Kanagawa 236, and **Central Institute for Experimental Animals, 1430 Nogawa, Miyamae-ku, Kawasaki-shi, Kanagawa 213, Japan*

(Received 13 March 1990/ Accepted 8 June 1990)

Eight inbred mouse strains were examined for their reactivity to the induction of antinucleolar autoantibody (AnuA) by mercuric chloride (MC). According to the positive ratio and titer of AnuA induced, the strains were grouped into high-, moderate-, low-, and non-responders. Since the susceptibilities to MC were different among strains having the same *H-2* haplotype, involvement of *H-2* unlinked gene (s) in AnuA induction by MC was suggested.

Mercuric chloride (MC) is well known to be a renal toxicant at high levels of exposure [1]. In addition, MC can induce autoimmunity by repeated exposure at low levels in rats [2, 14], rabbits [10], and mice [4, 8]. We previously reported that MC can induce antinucleolar autoantibody (AnuA) in outbred ICR mice but not in commercially available inbred mice such as BALB/c, CBA/J, C3H/He, C57BL/6, and DBA/2 [11]. However, it is impossible to elucidate the precise immunological mechanism(s) of AnuA induction by MC when outbred mice are used. We, therefore, investigated various strains of mice to establish a good experimental system. This brief note describes the results of AnuA induction by MC in 8 inbred strains.

IAI/Jcl, ICT/Jcl, IQI/Jcl, IPI/Jcl, and NOD/Jcl (CLEA Japan, Tokyo) which were derived from Jcl : ICR [5, 7], DDD/1 (Institute of Medical Science, Tokyo University, Tokyo), SJL/J (Gokita Breeding Service, Tokyo), and WHT/Ht (Yokohama City University, Yokohama) were examined. The sex and age of these mice are shown in Table 1. Mercuric chloride (Wako Chemical, Tokyo) was dissolved in 0.9% sterile saline, and the solution injected subcutaneously in mice at a dose of 1mg/kg 3 times a week for 4 weeks.

Mice were anesthetized with chloroform and then bled by heart puncture two days after the final injection. AnuA was examined by indirect immunofluorescence using R615B2 cells as substrate. Sera diluted 1 : 10 with phosphate buffered saline were screened and positive sera were further titrated by 2-fold dilution starting at 1 : 10. The antibody titer was expressed as the number of the tube with the highest dilution showing positive staining. Details of the methods have been described previously [11].

As shown in Table 1, a high-titer of AnuA was induced in all WHT/Ht and SJL/J mice. A high-titer of AnuA was also detected in all IQI/Jcl except for one female. While AnuA was detected in 11 of 12 IAI/Jcl mice, its titer was moderate. A low-titer of AnuA was detected in 3 of 15 DDD/1 and 9 of 21 ICT/Jcl. There was no significant sex difference in the positive ratio and titer of AnuA in ICT/Jcl, IQI/Jcl and WHT/Ht. AnuA was not induced in IPI/Jcl and NOD/Jcl. AnuA was not found spontaneously in control mice injected with only 0.9% saline in any strain (data not shown).

The present study clearly demonstrates that inbred mice can be grouped into high- (IQI/Jcl, SJL/J, and WHT/Ht), moderate- (IAI/Jcl), low- (ICT/Jcl and DDD/1), and non-responders (IPI/Jcl and NOD/Jcl) to MC according to the

Table 1. Antinucleolar autoantibody induced in inbred mice by mercuric chloride

Strain	Sex	Age (weeks)	Positive/Examined	Titer
DDD/1	F	6	3/15	2.0±1.0
IAI/Jcl	F	7	11/12	3.5±1.4
ICT/Jcl	F	7~17	3/13	1.0±0.0
	M	13~17	6/8	1.3±0.6
IQI/Jcl	F	6	8/9	7.1±0.6
	M	6	6/6	7.2±0.8
IPI/Jcl	F	6~14	0/9	
	M	9	0/9	
NOD/Jcl	F	7~8	0/6	
SJL/J	F	8	10/10	6.4±1.6
WHT/Ht	F	15	3/3	8.7±0.6
	M	15	5/5	8.5±0.5

Mercuric chloride was injected subcutaneously at a dose of 1 mg/kg 3 times a week for 4 weeks. The antibody titer was expressed as the number of the tubes with the highest dilution showing positive staining. Titer = mean ± SD

positive ratio and titer of AnuA induced. Although SJL/J was a high-responder, the strain is very difficult to get in Japan. It is also well known that breeding of the strain is extremely hard. On the contrary, IQI/Jcl (Taguchi, F. at CLEA Japan, personal communication) and WHT/Ht [13] breed well; therefore, these strains may be very useful to investigate the immunological mechanism(s) of AnuA induction by MC.

It has been reported that the major histocompatibility complex may be important in the induction of autoimmunity by chemicals [6] and that mice carrying *H-2s* and *H-2q* haplotypes are susceptible to induction of AnuA by MC [8,9]. The present result that IQI/Jcl (*H-2q*) and SJL/J (*H-2s*) were high-responders is in agreement with those reports, while the *H-2* haplotype of WHT/Ht is not yet known (Identification is in progress). On the other hand, it was remarkable that the susceptibilities to AnuA induction by MC were different between IAI/Jcl, ICT/Jcl, and IQI/Jcl (Table 1), while they have the same *H-2q* haplotype [5]. It was also noted that DDD/1, which is supposed to have *H-2s* [3], was a lowresponder. These observations suggested that resistance to AnuA induction by MC might be determined by a *H-2* unlinked gene(s). In fact, genetic studies have implicated the involvement of gene(s) other than the major histocompatibility com-

plex in regulating the induction of autoimmunity by MC in rats [12] and mice [9]. Genetic studies using IAI/Jcl, ICT/Jcl, and IQI/Jcl may be promising to detect the important *H-2* unlinked gene(s) regulating AnuA induction by MC.

The authors are indebted to Drs. K. Ueda and H. Iwai at the Institute of Public Health for their helpful discussion. They also thank the supervisors of each mousebreeding colony for providing the inbred mice. Thanks are also extended to Mrs. H. Kubota at the National Institute of Industrial Health for her technical assistance. This work was partly supported by a grant from the Ministry of Labor, Japan.

References

- [1] Berlin, M. (1979). In *Handbook on the Toxicology of Metals*. pp 503-530, Freiberg, L., Nordberg, G. F., and Vouk, V. B. edit., Elsevier Biomedical Press, Amsterdam.
- [2] Druet, P., Druet, E., Potdevin, F., and Sapin, C. (1970). *Ann. Immunol.*, **129** C, 777-792.
- [3] Fujiwara, M. (1987). In *New Methodology to Investigate the Lymphocyte Function*. pp 610-613, Yada, J. and Fujiwara, M. edit., Chugai-Igaku Press, Tokyo. (in Japanese)
- [4] Hultman, P. and Enestrom, S. (1987). *Clin. exp. Immunol.*, **67**, 283-292.
- [5] Kato, H. (1989). *Medical Immunol.*, **17**, 353-361. (in Japanese)
- [6] Kolb, J., Toyka, K. V., and Gleichmann, E. (1987). *Immunology Today*, **8**, 3-6.
- [7] Makino, S., Kunitomo, K., Muraoka, Y., Mizushima, Y., Katagiri, K., and Tochino, Y. (1980). *Exp. Anim.*, **29**, 1-13.

- [8] Reuter, R., Tessars, G., Vohr, H. W., Gleichmann, E., and Luhrmann, R. (1989). *Proc. Natl Acad Sci.*, **86**, 237-241.
- [9] Robinson, C. J. G., Balazs, T., and Egrov, I. K. (1986). *Toxicol appl Pharmacol*, **86**, 159-169.
- [10] Roman-Franco, A., Turiello, M., Albine, B., Milgrom, F., and Andres, G. A. (1978). *Clin Immunol Immunopathol*, **9**, 464-481.
- [11] Saegusa, J., Yamamoto, S., Iwai, H., and Ueda, K. (1990). *Ind. Health*, **28**, 21-30.
- [12] Sapin, C., Hirsch, F., Delaporte, J. P., Bazin, H., and Druet, P. (1984). *Immunogenetics*, **20**, 227-236.
- [13] Staats, J. (1985). *Cancer Res.*, **45**, 945-977.
- [14] Weening, J. J., Fleuren, G. J., and Hoedemaeker, Ph. J. (1978). *Lab. Invest.*, **39**, 405-411.

塩化水銀による抗核抗体の誘導

—マウス系統間の感受性差—

三枝 順三・木内 吉寛*・伊藤豊志雄**

労働省産業医学総合研究所

*横浜市立大学医学部

**財団法人実験動物中央研究所

DDD/1, IAI/Jcl, ICT/Jcl, IQI/Jcl, IPI/Jcl, NOD/Jcl, SJL/および WHT/Ht の 8 マウス系統について塩化水銀による抗核抗体の誘導を検索した。誘導された抗核抗体の陽性率および抗体価から高 (IQI/Jcl, SJL/J, WHT/Ht), 中 (IAI/Jcl), 低 (ICT/Jcl, DDD/1),

及び無反応 (IPI/Jcl, NOD/Jcl) 群に分類された。IAI/Jcl, ICT/Jcl, IQI/Jcl は何れも H-2 q ハプロタイプを有しているにもかかわらず感受性が異なっていたことから、抗核抗体誘導を制御する H-2 に連座しない遺伝子の存在が示唆された。