Effect of Nu/Nu Gene on Genetically Determined Resistance to Murine Cytomegalovirus

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SUMMARY

Adult athymic Nu/Nu mice showed increased susceptibility to lethal infection with murine cytomegalovirus (MCMV) when compared to their heterozygous T cell-competent Nu/+ littermates. However, the extent of this increase in susceptibility varied dramatically depending on the genetic background of the mice carrying the Nu/Nu gene. Genetically susceptible Balb/c (H-2<sup>d</sup>) mice showed a greater than 316-fold difference between the LD<sub>50</sub> of Nu/Nu and Nu/+ littermates. In marked contrast, the genetically resistant CBA (H-2<sup>k</sup>) strain was characterized by only a 16-fold difference in resistance between Nu/Nu and Nu/+ mice, and furthermore, the athymic CBA Nu/Nu mice were no more susceptible than the T cell-competent Balb/c Nu/+ strain. These results together with previous observations strongly suggest that the (H-2<sup>k</sup>)-associated resistance of the CBA strain is mediated by non-T cell-dependent early defence mechanisms.

The resistance of adult mice to lethal infection with murine cytomegalovirus (MCMV) is controlled by genes linked to the major histocompatibility complex (H-2 complex) of the mouse, as well as by non-H-2-linked genes [Chalmer et al., 1977; Grundy (Chalmer) et al., 1981]. Non-H-2 gene control of resistance to MCMV correlated well with activation of natural killer (NK) cells (Bancroft et al., 1981) and production of type I interferon [Grundy (Chalmer) et al., 1982]. NK cell-deficient beige mice (C57BL/6J bg<sup>+</sup>/bg<sup>+</sup>) showed increased susceptibility to the virus (Bancroft et al., 1981). However, the mechanism of the H-2-associated resistance remains unclear, although the genetic patterns observed [Grundy (Chalmer) et al., 1981] were similar to those controlling cytotoxic T cell responses in other virus and hapten systems, suggesting possible T cell involvement. Also, earlier studies had implicated an important role for T cell immunity in resistance to MCMV, since thymus-deficient (Nu/Nu) nude mice were more susceptible to the virus (Selgrade et al., 1976; Starr & Allison, 1977). The degree of increased susceptibility of Nu/Nu mice was a point of dispute: Starr & Allison (1977) reported a 100-fold difference in LD<sub>50</sub> whereas Selgrade et al. (1976) found only a 10-fold difference between Nu/Nu mice and their Nu/+ heterozygous littermates. We considered that perhaps this discrepancy was due to the genetic background of the nude mice examined. Starr & Allison (1977) had used CBA/CaCrc Nu/Nu mice but Selgrade et al. (1976) had not specified the background of their nude mice.

In order to establish the role of T cell-dependent immunity in genetically determined resistance to MCMV, we have examined the resistance of T cell-deficient Nu/Nu mice (compared to their T cell-competent heterozygous Nu/+ littermates) on three different genetic backgrounds: on the resistant CBA (H-2<sup>k</sup>) background and on that of two susceptible strains, Balb/c (H-2<sup>d</sup>) and C57BL (H-2<sup>b</sup>). Although the latter two strains are relatively susceptible, the C57BL strain is slightly more resistant to MCMV due to non-H-2-linked resistant genes [Grundy (Chalmer) et al., 1981].

CBA Nu/Nu, C57BL Nu/Nu and Balb/c Nu/Nu mice together with their heterozygous Nu/+ littermates were obtained from OLAC 1976 Ltd., UK, and were maintained in sterile isolation for the duration of these studies. All mice were females and aged 8 to 9 weeks. Mice
were inoculated intraperitoneally at the same time with the same stock of MCMV. The virus used was a virulent salivary gland homogenate [10 to 20% in medium 199 (Gibco) plus 10% foetal calf serum] prepared from weanling Balb/c mice inoculated 2 to 3 weeks previously with the Smith strain of MCMV [maintained in continuous passage in weanling mice as described previously (Chalmer et al., 1977)]. Virus stocks have been found to be free from contamination with the following viruses: ectromelia, polyoma, lymphocytic choriomeningitis, Sendai, mouse hepatitis, Reo-3, minute virus of mice, pneumonia virus of mice and murine encephalomyelitis virus. Control mice received normal salivary gland homogenate from the same shipment of mice as those used to prepare the virus stock. Experimental mice received half log dilutions of virus; 7 to 10 mice were used for each dilution.

The resistance to lethal MCMV infection of the various Nu/Nu and Nu/+ strains can be seen in Fig. 1. The LD₅₀ values have been plotted on a log₁₀ scale relative to the LD₅₀ of the Balb/c Nu/+ strain arbitrarily assigned a value of 1. The effect of the presence of the Nu/Nu gene in each strain was, as expected, to increase susceptibility; however, the extent of such an increase was dependent on the strain involved. On the CBA background the presence of the homozygous Nu/Nu gene resulted in a 16-fold increase in susceptibility compared to the heterozygous Balb/c Nu/+ littermates. In marked contrast, Nu/Nu mice on the Balb/c genetic background were greater than 316-fold more susceptible than their heterozygous littermates. Deaths occurred in all Balb/c Nu/Nu mice inoculated with the lowest dilution (1/316²) of virus used. Unfortunately, the number of C57BL Nu/Nu mice available was limited and an accurate LD₅₀ could not be established for this strain. However, no C57BL Nu/Nu mice died at any of the virus dilutions used which indicated that these mice must be at least as resistant as the heterozygous Balb/c Nu/+ strain. There were no deaths in any strain in control groups receiving normal salivary gland homogenate.

The time of death was substantially delayed in athymic Nu/Nu mice compared to Nu/+ heterozygotes. Deaths occurred between days 6 and 21 in the Balb/c Nu/Nu strain and from days 3 to 6 in the Nu/+ littermates. Nu/Nu mice on the CBA genetic background took even longer to die, deaths occurring between days 16 and 45 whilst Nu/+ heterozygote controls died on days 5 and 6. When compared at the Nu/Nu LD₅₀ virus dose or to a lesser extent at the Nu/+ LD₅₀ dose, the mean time of death of Nu/Nu mice was longer than that of Nu/+ controls.
The CBA (H-2^k) strain of mice is genetically resistant to MCMV because of both H-2 and non-H-2-linked genes [Grundy (Chalmer) et al., 1981; Bancroft et al., 1981]. As mentioned above the non-H-2-linked genetic control has been found to correlate well with activation of NK cells and production of type 1 interferon, whereas the mechanism of H-2-linked genetic control is not known. The present study shows that although the presence of the Nu/Nu gene reduces the resistance of CBA mice, these athymic CBA Nu/Nu mice are still no more susceptible than the T cell-competent normal Balb/c mouse (Fig. 1), strongly suggesting that the H-2-controlled as well as the non-H-2-controlled resistance of the former strain is probably mediated by non-T-cell-dependent early defence mechanisms. Balb/c (H-2^d) mice have both non-H-2- and H-2-linked genetic susceptibilities [Grundy (Chalmer) et al., 1981]. Assuming that both these defects relate to non-T-cell-dependent resistance mechanisms, then if T-cell immunity is also lacking, as in the athymic Balb/c Nu/Nu mouse, there would be little if any resistance left against the virus. Our findings here that Balb/c Nu/Nu mice are indeed extremely sensitive to the lethal effects of MCMV lend support to this assumption. However, the hypothesis that H-2-linked genetic control of resistance is non-T-cell-dependent can only be proven by examining the resistance of Nu/Nu mice on the Balb.K (H-2^k) genetic background, as these mice have the H-2-associated resistance of the k haplotype (as in CBA mice) but the non-H-2-linked susceptibility of the Balb/c genetic background. Such mice are not available at present.

CBA Nu/Nu mice succumb to the lethal effects of MCMV only late in infection and to large doses of virus presumably because final elimination of virus-infected cells, normally effected by T cells, fails to take place. At early stages of infection, competent immediate defence mechanisms are operative in CBA Nu/Nu mice which are adequate to protect against all but the largest doses of virus, and indeed deaths at these doses are substantially delayed. Part of this early defence to MCMV appears to be mediated by activation of NK cells (Bancroft et al., 1981), a mechanism which is more efficient in mice of the CBA than the Balb/c genetic background.

However, athymic nude mice have often been reported to exhibit high levels of NK cells when compared to their heterozygous littermates (Herberman & Holden, 1978) and previous studies from our group have indeed shown enhanced levels of NK activation following MCMV infection of Balb/c Nu/Nu mice compared with Nu+ controls (Bancroft et al., 1981). One might therefore have expected that the Balb/c Nu/Nu mouse would show increased resistance to the virus compared to the Balb/c Nu/+ heterozygote (normally poor in such NK activation). The present study showed clearly that this was not the case, although possibly the better NK response of Balb/c Nu/Nu mice contributed to the delay in deaths in this group compared to Nu/+ controls at a given virus dose. One must therefore assume that NK activation is not by itself sufficient to ensure protection against lethal infection and that additionally important factors present in CBA Nu/Nu mice are still deficient in the Balb/c Nu/Nu strain. Again, these findings support the hypothesis that the H-2^k-associated resistance of CBA mice is non-T-cell-dependent.

The disparate reports of the susceptibility of Nu/Nu mice to MCMV in earlier literature (referred to above) may well be reconciled when genetic constitutions of the mice used are examined. However, our finding here that the LD50 of CBA Nu/Nu mice is 16-fold lower than that of CBA Nu/+ mice conflicts with the 100-fold difference reported previously in that strain by Starr & Allison (1977). In this instance, another important factor in determining resistance to MCMV, namely age, most likely accounts for the disparity. Resistance to MCMV rises rapidly in the fourth week of life (Booss & Wheelock, 1975) and subsequently continues to rise gradually, stabilizing at 8 to 10 weeks of age [J. E. Grundy (Chalmer) et al., unpublished observations). In our study care was taken to use age-matched animals which were 8 to 9 weeks old at the time of inoculation. In contrast, the nude mice used in the study...
Short communications

reported by Starr & Allison (1977) were 4 to 6 weeks old, a time at which resistance, especially of the immediate type, has not reached full maturity, and at which the resistances of individual mice varied considerably. It is not unlikely, therefore, to find that such immature nude mice were more susceptible than the 8- to 9-week-old mice used in the present study.

In conclusion, the role of T-cell-mediated immunity in defence against lethal MCMV infection was found to vary dramatically depending on the strain of mice, being crucial in genetically susceptible Balb/c (H-2d) mice and of lesser importance in genetically resistant CBA (H-2k) mice. The results presented here again demonstrate the dramatic effect of host genetic constitution on the outcome of infection with MCMV, and emphasize the importance both of carefully documenting the strain used, and of using a variety of strains of mice when investigating the pathogenesis of this virus infection.

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REFERENCES


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