Polyadenylic:polyuridylic acid-induced protection of BALB/c mice against acute murine cytomegalovirus infection

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Treatment of BALB/c mice with poly(A):poly(U) 18 h prior to infection with a lethal dose of murine cytomegalovirus (MCMV) increased survival. In parallel with increased survival, a 10- to 100-fold reduction of plaque-forming MCMV was found in the liver and spleen of mice 4 days post-infection with a sublethal dose of MCMV. Poly(A):poly(U) did not significantly increase natural killer cell activity or prolong the duration of elevated cytotoxic activity in infected animals. The possible role of interferon in the poly(A):poly(U)-induced protection of BALB/c mice is discussed.

Primary herpesvirus infections are normally self-limiting and symptoms, when expressed, usually resolve without intervention. However, following recovery, some virus will remain in the host in a latent state from which secondary infections can be induced (Stevens, 1989). Cytomegalovirus (CMV), like other herpesviruses, can assume a latent state after primary infection (Jordan, 1983). Although activation of a latent CMV infection may occur periodically, it is only among immunocompromised patients that productive CMV replication is associated with increased morbidity and mortality (Ho, 1982; Jacobson & Mills, 1988).

With the increasing numbers of immunocompromised individuals there is need to control both primary and secondary herpesvirus infections. Anti-herpetic drugs are available, but toxicity and the risk of selection of drug-resistant virus variants prevent their routine prophylactic use (Balfour, 1990; Drew et al., 1990). Passive immunization has been used to protect high risk patients against primary infection (Snydman, 1990). Clinical evidence suggests that CMV infections are controlled by cell-mediated immune mechanisms (Quinnan et al., 1982a), and treatments aimed at reconstituting these functions may help in the management of CMV disease.

A number of immunomodulating substances, able to protect non-specifically against a variety of infectious agents including viruses, bacteria and fungi, have been described. One such adjuvant, polyadenylic : polyuridylic acid [poly(A):poly(U)] is a non-toxic, synthetic dsRNA that has been shown to have immunomodulatory effects on both humoral and cellular immune responses (Johnson, 1979). In human clinical trials, poly(A):poly(U) has been found to be well tolerated and is reported to increase long-term survival following surgical removal of operable breast and stomach tumours (Lacour et al., 1980; Youn et al., 1990). Stimulation of natural killer (NK) cell activity appears to explain, in part, the adjuvant effect of poly(A):poly(U) on tumour therapy (Youn et al., 1987).

Murine CMV (MCMV) infection of mice closely parallels the pathogenesis of human CMV disease and provides an experimental model for in vivo CMV investigations (Osborn, 1982). Inbred mouse strains differ in their susceptibility to MCMV such that the lethal dose for a resistant strain such as C3H can be 25-fold that needed to kill highly susceptible BALB/c mice (Bancroft et al., 1981). Fig. 1 demonstrates that poly(A):poly(U) treatment of BALB/c mice provided significant protection against infection with virulent, salivary gland-passaged MCMV (Smith strain). All 11 mice in the unprotected control group succumbed between 4 and 6 days post-virus infection. Of the 21 mice receiving either 150 μg or 300 μg of poly(A):poly(U) prior to infection, 14 survived intraperitoneal injection of \(3 \times 10^4\) p.f.u. MCMV. Moreover, deaths occurred later in the poly(A):poly(U)-treated animals.

The ability of poly(A):poly(U) to protect is MCMV dose-dependent. Thus, 100% of BALB/c mice injected with 300 μg of poly(A):poly(U) 18 h prior to infection with \(3 \times 10^3\) p.f.u. MCMV survived, whereas only three of 10 polynucleotide-pretreated animals survived infection with \(1 \times 10^5\) p.f.u. of virus. A few unprotected
Short communication

Survival following MCMV infection has been shown to be related to the virus titre in certain target organs. Thus, early after infection less virus is found in the liver and spleen of genetically resistant strains such as C3H (Mercer & Spector, 1986). A comparison of the virus titres in spleen and salivary glands of poly(A):poly(U)-treated and untreated BALB/c mice at various times following infection with $3 \times 10^3$ p.f.u. MCMV is shown in Fig. 2.

Two days post-infection, virus had replicated and colonized splenic tissue of all animals, although somewhat more infectious virus was seen in organs taken from the control mice at this time (Fig. 2). The spleens of the control mice were smaller on day 2 than those of the poly(A):poly(U)-treated animals (60 and 70 mg compared to 130 and 130 mg respectively). If calculated as total virus/organ (rather than per ml of 10% tissue homogenate), there was still about sixfold the amount of virus in the spleens of the unprotected mice.

Virus continued to replicate in the spleens of mice that had received only PBS prior to infection, with a peak titre observed on day 4. In contrast, the titre of virus recovered from the spleens of poly(A):poly(U)-treated animals remained nearly constant between days 2 and 6. On day 4 there was 100-fold the amount of MCMV in the spleens of the unprotected mice. From day 4 onward, the spleens of the poly(A):poly(U)-treated and untreated groups were comparable in weight and the differences were not due to variation in organ size.

Virus had been cleared from the spleen of three of the four mice killed on day 8 and the data for the unprotected control animals are thus comparable to those reported for BALB/c mice by Mercer & Spector (1986). As described by these workers, virus could be detected in the spleen of an occasional BALB/c mouse after day 8. However, recovery of virus at later times was sporadic and unrelated to whether the animals had received poly(A):poly(U).

Virus also colonized the liver of both poly(A):poly(U)-treated and untreated animals by day 2. As with spleen, the liver MCMV titres at days 4 and 6 were significantly lower in animals that had received poly(A):poly(U) pretreatment. Virus spread to the salivary glands as it was cleared from the liver and spleen in both the control and poly(A):poly(U)-treated animals. The titre of the control mice (three of 20 infected animals; Fig. 2) succumbed following infection with $3 \times 10^3$ p.f.u. of the same MCMV stock. It appeared that animals treated with poly(A):poly(U) could survive roughly a 10-fold greater MCMV challenge when polynucleotide was given 18 h prior to infection. The time of poly(A):poly(U) treatment was important and little protection was achieved when the polynucleotide complex was given either 48 h prior to virus or 24 h post-infection.
virus in the persistently infected salivary glands remained nearly constant in all animals over the duration of the experiment. The 10-fold fluctuations in titres between alternate sampling days is attributed to minor variations in assay conditions and the use of different stocks of mouse embryo fibroblasts (MEFs) on titre plates. Thus, poly(A):poly(U) did not reduce the amount of virus in persistently infected salivary glands. Mercer & Spector (1986) have reported that virus titres in salivary glands of BALB/c mice decrease to undetectable levels sometime between 3 and 4 weeks post-infection. In other experiments, we measured virus titres 2 to 4 months post-infection and confirmed that infectious MCMV eventually could not be recovered from the salivary glands of infected mice. Whether virus was present in a latent state was not examined.

Since death of untreated BALB/c mice occurred before day 6 (Fig. 1), the protective mechanism(s) evoked must operate within the first few days post-infection. The titre of MCMV in splenic tissue of different mouse strains 3 days post-infection has been shown to be inversely correlated with survival (Quinnan & Manischewitz, 1987). The reduced titres of MCMV recovered from liver and spleen of the poly(A): poly(U)-treated animals (Fig. 2) are most probably related to survival. It is noted that for the highly resistant C3H strain, the titres are not only lower but virus is cleared from both liver and spleen by day 6 (Mercer & Spector, 1986), 2 days earlier than seen here for BALB/c mice.

NK cells are known to provide protection against lethal MCMV infection in many mouse strains, including the highly resistant C3H mouse. The evidence indicating the importance of NK cells to survival includes (i) the NK cell response induced following MCMV infection is, with a few exceptions, directly related to the resistance of the mouse strain (Bancroft et al., 1981), (ii) NK cell activity is maximal early after infection when protection is required (Bancroft et al., 1981; Quinnan et al., 1982b), (iii) beige mutant mice, which are deficient in NK cells, are more susceptible to MCMV infection than heterozygous litter mates (Shelam et al., 1981), (iv) reduction of NK cell numbers by treatment with either anti-asialo or monoclonal anti-NK 1.1 antibodies lowers the resistance to MCMV (Bukowski et al., 1984; Shanley, 1990), and (v) purified splenic NK cells from adult mice can passively confer resistance to MCMV infection to neonatal mice which are developmentally deficient in NK cell activity (Bukowski et al., 1985). Accordingly, the NK cell response of BALB/c mice to MCMV infection was investigated. The cytotoxicity of spleen cells isolated at various times after either mock or MCMV infection was measured by chromium release from YAC-1 target cells (Odean et al., 1991). Three different spleen:target cell ratios (100:1, 50:1 and 25:1) were employed. Data for one ratio, 50:1, are summarized in Fig. 3.

Only low levels of cytotoxicity were detected in mice injected with PBS. Background cytotoxicity was similar for animals receiving an injection of PBS 18 h prior to either being infected with 4 × 10³ p.f.u. MCMV or being mock-infected with PBS. Animals were killed at the times indicated relative to the time of infection and single-cell suspensions were prepared from individual spleens. Splenic effector cells were mixed with ¹¹⁵Cr-labelled YAC-1 cells at different ratios and the mixtures were incubated at 37°C for 4 h. Chromium release was measured and percentage cytotoxicity calculated as [c.p.m. (experimental) − c.p.m. (spontaneous)/c.p.m. (maximum) − c.p.m. (medium)] × 100. The data measured at an effector: target cell ratio of 50:1 are plotted in bar graph form; the standard error of the mean is indicated.

Fig. 3. Duration of NK cell response after stimulation with poly(A): poly(U), MCMV or both polynucleotide and virus. Male BALB/c mice were injected with either 300 μg poly(A):poly(U) in PBS or just PBS prior to either being infected with 4 × 10³ p.f.u. MCMV or being mock-infected with PBS. Animals were killed at the times indicated after either treatment with either anti-asialo or monoclonal anti-NK 1.1 antibodies lower the resistance to MCMV (Bukowski et al., 1984; Shanley, 1990), and (v) purified splenic NK cells from adult mice can passively confer resistance to MCMV infection to neonatal mice which are developmentally deficient in NK cell activity (Bukowski et al., 1985). Accordingly, the NK cell response of BALB/c mice to MCMV infection was investigated. The cytotoxicity of spleen cells isolated at various times after either mock or MCMV infection was measured by chromium release from YAC-1 target cells (Odean et al., 1991). Three different spleen:target cell ratios (100:1, 50:1 and 25:1) were employed. Data for one ratio, 50:1, are summarized in Fig. 3.

Only low levels of cytotoxicity were detected in mice injected with PBS. Background cytotoxicity was similar for animals receiving an injection of PBS 18 h prior to killing at time 0 and those receiving a second injection of PBS at time 0 and being killed 1, 2 or 4 days later (data not shown). Poly(A):poly(U) alone induced some NK cell activity. The response induced by polynucleotide was highest 18 h after treatment and decayed to the baseline level 2 days after mock infection with PBS at time 0.

MCMV infection stimulates cytotoxic activity measurable by chromium release from YAC-1 cells. The NK cell activities measured on both days 1 and 2 following MCMV infection were similar for both poly(A):poly(U)-treated and untreated animals. Prior stimulation of NK cell function with poly(A):poly(U) apparently did not inhibit subsequent virus-induced stimulation of NK cell function. By day 4 most of the cytotoxicity measured
by the assay had decayed. The low cytotoxicity at day 4 in unprotected BALB/c mice is in agreement with published data (Bancroft et al., 1981).

The conditions of poly(A):poly(U) treatment and virus infection were selected to simulate those used for the virus titre studies shown in Fig. 2. The cellular cytotoxicity should reflect levels present at times when virus replication was restricted in poly(A):poly(U)-treated animals. Whereas the poly(A):poly(U)-treated mice had a somewhat higher residual NK cell activity 4 days after infection, the level was low, suggesting that other functions are acting to restrict MCMV replication in BALB/c mice. In C3H mice, the NK cell activity induced by MCMV is high from days 2 to 5 post-infection (Bancroft et al., 1981). It is not until day 9 that the residual NK cell activity in C3H mice decays to a level comparable to that measured for poly(A):poly(U)-treated BALB/c mice on day 4.

A variety of mechanisms probably operate in vivo to protect various mouse strains against acute MCMV infection. Genetic determinants both linked and unlinked to the H-2, or major histocompatibility, locus of the mouse contribute to the resistant phenotype of different strains. Thus, congenic BALB/k mice, which have the same H-2d haplotype as C3H mice, are about 10 times more resistant than BALB/c mice, which are H-2d (Bancroft et al., 1981; Quinnan & Manischewitz, 1987). The sensitive phenotype completely dominates in F1 hybrids between BALB/c and BALB/k mice (Quinnan & Manischewitz, 1987). Similar crosses between BALB/c and C3H mice produce progeny which are nearly (Quinnan & Manischewitz, 1987), if not equally (Grundy (Chalmer) et al., 1981) as susceptible to MCMV as BALB/c mice. Mice with a C57BL background have a resistant phenotype involving non-H-2-linked determinants which dominates in crosses with BALB/c mice [Grundy (Chalmer) et al., 1981; Quinnan & Manischewitz, 1987]. The mechanisms of protection associated with the various resistance determinants have only been partially characterized.

High NK cell activity is not universally associated with resistance to MCMV. B10.BR mice, which have the H-2d histocompatibility type expressed on a C57BL genetic background, are as resistant to MCMV infection as C3H mice However, the splenic NK cell activity augmented by MCMV is as low in the B10. Br strain as in BALB/c mice (Bancroft et al., 1981). Because poly(A):poly(U) induces a marginally greater NK cell response of only limited duration, it appears that other cells or protective mechanisms contribute to the resistant phenotype of the treated BALB/c mice.

Interferon (IFN) as well as IFN inducers, including double-stranded polyribonucleotides, augment the NK cell activity of different mouse strains (Djeu et al., 1979; Youn et al., 1983). The resistant phenotype associated with the H-2d genotype is IFN-mediated, although the factor(s) controlling IFN levels are, themselves, unlinked to the H-2 loci [Grundy (Chalmer) et al., 1982]. Thus, anti-IFN antibodies block stimulation of NK cell activity and increase the sensitivity of C3H mice to MCMV (Djeu et al., 1979). The dominant MCMV sensitivity associated with the H-2d genotype of BALB/c mice is, in contrast, unaffected by IFN. It is not possible to protect BALB/c mice with exogenous IFN and anti-IFN antibodies do not increase further the sensitivity of this strain [Grundy (Chalmer) et al., 1982]. Organ IFN titres are equivalent in sensitive (BALB/c) and resistant (C3H) strains 48 h post-infection with a sublethal dose of MCMV (Allan & Shellam, 1985), and IFN production does not seem critical to survival. Moreover, other IFN inducers, including endotoxin and Bacille Calmette-Guérin, do not protect BALB/c mice (Chalmer et al., 1977). The IFN-inducing potential of poly(A):poly(U) may thus be of limited importance in protection of BALB/c mice against MCMV infection. Kern et al. (1978) have reported that another synthetic polyribonucleotide complex, polyinosinic:polycytidylid acid, protects 6-week-old Swiss Webster mice against MCMV when injected 6 to 18 h before virus. As is the case with BALB/c mice, these workers were unable to achieve protection with exogenous type I IFN. We have not, as yet, investigated whether poly(A):poly(U), which is devoid of the toxicity of other synthetic dsRNAs, will protect other mouse strains.

References


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