The envelope of Mason–Pfizer monkey virus has immunosuppressive properties

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We have demonstrated previously that the envelope protein of a murine retrovirus, Moloney murine leukaemia virus, has immunosuppressive properties in vivo. This property was manifested by the ability of the protein, when expressed by tumour cells normally rejected by engrafted mice, to allow the env-expressing cells to escape immune rejection and to proliferate. Here, it is shown that this property is not restricted to the envelope of a murine retrovirus, but is also shared by the envelope encoded by a primate retrovirus, Mason–Pfizer monkey virus.

Mason–Pfizer monkey virus (MPMV) is a primate retrovirus that was first detected by electron microscopy in a mammary carcinoma of a female rhesus monkey (Chopra & Mason, 1970; Jensen et al., 1970), from which it was molecularly cloned and characterized (Barker et al., 1986; Sonigo et al., 1986). Yet, while some initial studies suggested that the virus could transform a number of simian cell lines (Fine et al., 1974), the association with cancer has proved fleeting. Rather, newborn rhesus monkeys experimentally inoculated with this prototypic D-type retrovirus develop a wasting disease within a few weeks that is accompanied by opportunistic infections including pneumonia, enteritis and rashes (Fine et al., 1975; Bryant et al., 1986). While the virus was widespread, post-mortem examination revealed only lymphadenopathy and thymic atrophy (Fine et al., 1975). The coincidence of opportunistic infections and a thymic target suggested that the virus may exert a T cell-immunosuppressive effect, as has been suggested for other D-type retroviruses (Fine et al., 1975; Arthur et al., 1986; Bryant et al., 1986). Infection by retroviruses is frequently associated with immune cell dysfunction. In the case of bona fide oncogenic retroviruses [e.g. feline leukaemia virus (FeLV) and Moloney murine leukaemia virus (MoMLV)], it was strongly suggested that their envelope proteins have ‘immunosuppressive’ effects, based essentially on a series of in vitro assays involving lymphocyte proliferative responses to mitogens (reviewed in Oostendorp et al., 1993; Haraguchi et al., 1997; see also Mathes et al., 1979; Cianciolo et al., 1980; Mangeney & Heidmann, 1998). Here, we show that the MPMV envelope is actually immunosuppressive in vivo, using an assay involving rejection of tumour cells in immunocompetent mice, which further suggests that the MPMV envelope can inhibit the T cell response.

To assay the immunosuppressive activity of the MPMV envelope, we used the procedure that we devised previously (Mangeney & Heidmann, 1998), which is illustrated in Fig. 1(a). Murine tumour cells (MCA205 and CL8.1 cells; see below) were transfected with an MPMV-env expression vector (pTMO; Brody et al., 1994) (or an empty expression vector) and antibiotic-resistant cell populations were isolated. These cells expressed the stably transduced envelope vector, as illustrated in the RT–PCR analysis shown in Fig. 1(b). The cells were then engrafted into immunocompetent mice. In a first series of experiments, we used methylcholanthrene-induced murine fibrosarcoma cells (MCA205; H-2b haplotype). Control tumour cells, when injected into an allogeneic host (five to ten BALB/c mice per group; H-2b haplotype), led to the development of small tumours, in only a limited number of engrafted animals (0–25%: Fig. 1c, bottom). Under the same conditions, the MCA205 cells expressing the MPMV envelope were able to form easily detectable tumours that persisted for at least 2 weeks in a large proportion of the engrafted animals (> 80%; Fig. 1c, top). This enhancement of tumour cell growth was not observed, under identical experimental conditions, with ‘irrelevant’ expression vectors encoding transmembrane proteins unrelated to retroviral envelopes (the murine CD2 and erythropoietin receptor proteins; data not shown) (Mangeney & Heidmann, 1998). Induction of tumour formation was not due to any difference in intrinsic growth rates between the control and MPMV envelope-transduced cells, as tumour development induced by the two cell populations was identical when engrafted into a syngeneic host (C57BL/6, H-2b haplotype) (Fig. 1c, insets).

In a second series of experiments, we used the CL8.1
Fig. 1. (a) Rationale of the in vivo assay for the immunosuppressive, tumour-inducing effect of the MPMV envelope. The pTMO env-expressing vector containing the neomycin resistance gene (neo) has been described previously (Brody et al., 1994), as have the cell types and experimental protocol used (Mangeney & Heidmann, 1998). (b) RT–PCR of RNAs isolated from MPMV-env stably transduced cells, with control PCR on pTMO (vector) DNA, using the primers indicated in (a). RT–PCR (with and without an RT step) was performed by standard procedures using 1 µg total RNA. (c)–(d) In vivo assay for suppression of the immune response to env-transduced tumour cells in allogeneic (c) and syngeneic (d) contexts. Cells transduced with the MPMV env-expression vector (top) or without the env gene (bottom) were injected subcutaneously into immunocompetent mice at day 0. Occurrence of tumours and tumour sizes were then determined twice or thrice weekly. The percentages of animals with tumours are shown by filled bars (five to ten mice per group); mean tumour areas over 1 mm² are indicated by open bars. Insets: in vivo control growth of env-transduced and control cells in C57BL/6 syngeneic mice for the MCA205 cells (c) and in X-ray irradiated (500 rads) C57BL/6 mice for the CL8.1 cells (d).
tumour cell line (H-2\textsuperscript{b} haplotype) to address a physiologically more relevant situation. Injection of these tumour cells into a syngeneic host (five to ten C57BL/6 mice per group; H-2\textsuperscript{b} haplotype) led to the formation of small tumours, again in only a limited fraction of the engrafted animals (0–20%; Fig. 1, bottom), although they grew into large tumours in all cases when the mice were rendered immunodeficient by prior X-ray irradiation (see below). With MPMV envelope-transduced CL8.1 cells, we observed that expression of this envelope also severely enhanced tumour growth in mice (Fig. 1, top); a large fraction of the immunocompetent mice (>80%) developed tumours, which grew continuously, leading to death. Again, tumour growth was not due to intrinsic differences in rates of proliferation between the envelope-transduced and control cells, as similar profiles were observed for the two cell types when engrafted into X-irradiated hosts (Fig. 1, insets). Clearly, expression of the MPMV envelope gene, as demonstrated previously for the MoMLV envelope (Mangeney & Heidmann, 1998), enables the transfected tumour cells to escape immune rejection in immunocompetent mice in both an allogeneic and a syngeneic context.

Previous experiments, using synthetic peptides \textit{in vitro} (Cianciolo \textit{et al.}, 1985; reviewed in Oostendorp \textit{et al.}, 1993; Haraguchi \textit{et al.}, 1997), have suggested that a well-conserved domain of 17 amino acids, located within the transmembrane (TM) subunit of retroviral envelopes, might be responsible for the immunosuppressive effects. This domain can also be found in the MPMV envelope, at a position and with a sequence related to that of the well-characterized MoMLV sequence (yet with a five amino acid difference, see Fig. 2(a)). In order to analyse the possible role of this domain in the \textit{in vivo} immunosuppressive effect of the MPMV envelope, we used the D33 mutant MPMV envelope, constructed and characterized by Brody & Hunter (1992), which has 11 amino acids deleted from this domain (Fig. 2(a)). A remarkable feature of this construct is that the D33 deletion has no deleterious effect on the levels of protein synthesis and processing, the only major difference from the wild-type envelope being a loss of interaction between the surface (SU) and TM subunits of the envelope, resulting in the release of SU from the cell surface into the culture medium (Brody & Hunter, 1992; Brody \textit{et al.}, 1994; and data not shown). Accordingly, an expression vector identical to the one for the wild-type MPMV envelope of the assay shown in Fig. 1 was used to transduce murine tumour cells with the mutant D33 envelope and to assay for tumour growth in mice. Interestingly, as illustrated in Fig. 2(b), the

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Fig. 2. (a) Delineation of the deletion within the D33 mutant MPMV envelope, and comparison with the corresponding domain of the wild-type (wt) MPMV and MoMLV envelopes (sequence of the CKS-17 peptide in bold; Cianciolo \textit{et al.}, 1985). (b) \textit{In vivo} assay for suppression of the immune response to MCA205 cells transduced with the wild-type MPMV envelope (filled bars), the D33 deleted MPMV envelope (shaded bars) and controls (open bars). The experimental conditions were the same as in the experiment shown in Fig. 1(c), with the percentages of animals with tumours indicated (five to ten BALB/c mice per group).
D33 mutant envelope still showed an immunosuppressive effect in the in vivo assay, although to a reduced extent compared with the wild-type envelope. This result indicates clearly that the so-called ‘immunosuppressive’ domain is not the sole domain implicated in the in vivo immunosuppressive effect. This favours the involvement of several domains within the envelope protein, possibly dependent upon the overall structure of the protein. This would be consistent with the previously mentioned importance of peptide conformation for reported in vitro effects (Monell & Strand, 1994), as well as for the much higher efficiency of complete envelope proteins versus peptides in these assays (Oostendorp et al., 1993). Delineation of the effective domains involved in the immunosuppressive effect will probably be difficult, taking into account also that mutations within the TM moiety of retroviral envelopes are often deleterious for protein synthesis, stability or export (e.g. Brody & Hunter, 1992; Brody et al., 1994).

The ability of type-D retroviruses to suppress the host immune response is well documented. In the case of SRV-1, a naturally infected in primate centres and zoos die from experimentally inoculated with MPMV as well as macaques (Oostendorp et al., 1993). The present results therefore provide hints for the physiopathology of MPMV and further suggest strongly that the env gene per se could be the central, and possibly the sole, effector of immunosuppression. In fact, immunosuppression is likely to be a general strategy of retroviruses, also used by other pathogens, allowing efficient invasion of the host (see Oostendorp et al., 1993; Haraguchi et al., 1997; Naniche, 2000). Our data also suggest that MPMV might be involved secondarily in a tumorigenic process, associated with a decreased efficiency of the immune system in the elimination of naturally occurring transformed cells. This could account for the fact that the MPMV was first isolated from a breast tumour (Jensen et al., 1970; Chopra & Mason, 1970) under conditions where no other retrovirus could be detected (Bryant et al., 1986), although MPMV is clearly not an oncogenic retrovirus sensu stricto.

Sandra Blaise and Marianne Mangeney contributed equally to this work. We thank Dr E. Hunter for the gift of the pTMO plasmids expressing the wild-type and D33 mutant MPMV envelopes. This research was supported by a grant from the Ligue Contre le Cancer (‘Equipe Labellisée’).

References


Received 25 August 2000; Accepted 7 March 2001