Adoptive T cell immunotherapy of MSV-induced tumours in nude mice. Part I. Biology of tumour regression and recurrence

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The induction of immunity to progressively growing murine sarcoma virus (MSV) tumours in nude (nu/nu) mice by reconstitution with immune T cells from syngeneic (+/+) donors has been studied. Whole spleen cell preparations served as the source of immune T cells. Transfer of immune, but not of normal, spleen cells resulted in partial or apparently complete regression of primary tumours and a related moderate to considerable extension of survival time. The dose, the time in days between immunization and transfer, as well as timing of the spleen cells in relation to tumour cell challenge, were all factors which influenced the effectiveness of the protective inocula. An unexpected consequence of even the very effective primary immunotherapy regimens, was secondary tumour development after varying tumour-free intervals. This was most frequently manifest as tumour recurrences at the original injection site either on their own or in combination with distant metastases. Such a relatively high frequency of tumour reappearance and metastatic spread contrasts markedly with the rare instances of secondary regrowth in normal immunocompetent mice. The present reconstitution system may therefore provide a new model for studying the inhibitory or stimulatory properties of T cells with respect to tumour regression and dissemination.

Abbreviations

nu/nu  Homozygous nude mice on a BALB/c background
+/+  Homozygous normal BALB/c mice
SPF  Specific pathogen free
CR  Complete regression of primary tumour

Introduction

Inoculation of adult immunocompetent mice with the Moloney strain of murine sarcoma virus (MSV), or MSV-induced tumour cells, results in the rapid appearance of local tumours which are generally rejected within 2–4 weeks of injection (reviewed in [11]). Tumour rejection is associated with the development of anti-tumour immunity, mice that have rejected the primary lesion (regressor mice) being resistant to a second MSV challenge [5, 19]. The tumours themselves are composed of both sarcoma cells and an inflammatory cell infiltrate [11], and tumour specific immune responses by macrophages [12, 25] and lymphocytes [12] extracted from the tumours have been demonstrated in vitro.
Both serum and spleen or lymph node cells from regressor mice protect normal and immunologically compromised recipients from tumour challenge (reviewed in [17]). Adoptive cellular immunotherapy is effective if given 4 days before [27], at the time of virus challenge [8], or up to 4 days later [15]. Removal of T cells from regressor spleen populations abolishes protection [8], and there is some evidence that protection of immuno-suppressed recipients is mediated by T cells with helper phenotype [14]. Regressor T cells recognize virus-coded antigens in vitro [13], and normal T cells immunized with purified virus in vitro are protective on adoptive transfer [9].

In newborn mice, inoculation with MSV causes progressive and lethal tumour growth [11]. The tumours in newborn animals are composed of tumour cells only, without any mononuclear cell infiltrate [18]. Similar progressive tumour growth is seen in sublethally irradiated and in neonatally thymectomized mice, in lethally irradiated adult mice reconstituted with bone marrow, and in animals treated with anti-thymocyte serum (reviewed in [17]). Tumour regression can be induced in these immunologically deficient hosts by adoptive transfer of regressor T cells [17, 2]. Although occasional recurrence of MSV tumours in individual normal animals has been noted [11], the reconstitution of mice rendered immunodeficient artificially has not been reported to predispose to tumour recurrence and/or metastasis.

In congenitally athymic, nude mice, injection of MSV virus also leads to progressive tumour growth with death of the animal [1]. Some workers have noted an increased latent period between virus inoculation and tumour development in nude mice [4, 23]. Tumour regression can, however, be achieved by implantation of syngeneic thymus before virus injection [24].

In the present study we have compared the survival times and tumour status of nude (nu/nu) mice injected with MSV tumour cells alone, with that of mice receiving syngeneic +/+ regressor spleen cell immunotherapy. Reconstitution promotes tumour regression and prolongs survival when immune, but not normal, spleen cells are transferred. Adoptive immunotherapy delayed until tumours are palpable remains effective in producing complete tumour regression. A majority of the reconstituted regressor mice, however, develop local tumour recurrence, often with metastatic secondary sarcoma, after variable tumour-free periods. These experiments suggest that although single shot cellular immunotherapy can effectively arrest primary tumour growth, it often has the unexpected consequence of later recurrence and metastatic spread, a secondary development rarely seen in normal immunologically competent mice.

Materials and methods

Mice
Nude (nu/nu) mice on a BALB/c background were bred at the ICRF Animal Unit, Mill Hill, London NW7, using outbred nude mice (LAC, Carshalton) and a backcross--intercross production system. Young adult mice of at least 11 BALB/c backcross generations, were used in the present experiments. SPF neonatal (<10 days old), and adult BALB/c +/+ mice were also obtained from the ICRF Animal Unit. Neonatal mice were transported together with their mothers in filter boxes and transferred on arrival to the Isolator (Pathoflex, Vickers Medical) which also housed the nude animals.