Lactate dehydrogenase-elevating virus induces antibodies reactive with a surface antigen of aetiologically unrelated murine cell transformants

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Mice infected with lactate dehydrogenase-elevating virus (LDV) developed antibodies reactive with a tumour cell surface antigen (TSA) of Moloney sarcoma virus (Mo-MSV)-transformed mouse cells (Sac). We demonstrate that STU mice infected with LDV were protected against growth of syngeneic Sac tumour cells as early as 23 days post-infection (p.i.) and up to 5 months p.i. Nine LDV strains, including the neurovirulent LDV-C, elicited production of anti-TSA antibodies, which were restricted to the IgM isotype. Monoclonal anti-TSA antibodies were raised 4 days after infection of STU mice with LDV. When tested against several transformants of STU and BALB/c mouse origin they were found to react with Mo-MSV transformants (PV-TC-77, STU mouse origin; MSV85 C1 3, BALB/c mouse origin), methylcholanthrene-transformed MethA cells (BALB/c origin) and L929 cells. We suggest that the well known tumour growth inhibition by LDV is due to LDV-induced anti-TSA antibodies.

Lactate dehydrogenase-elevating virus (LDV), currently classified as a togavirus, causes persistent viraemia in mice and is known to alter both antibody and cell-mediated immune reactions. The virus was detected 30 years ago as a contaminant of various murine tumours (Riley et al., 1960) and has been shown to influence their growth (for review see Rowson & Mahy, 1985). Depending upon the timing of LDV infection, stimulation or depression of tumour development was observed: depression occurred in chronically infected mice and stimulation early after infection (Michaelides & Schlesinger, 1974). However, an aetiological link between LDV infection and tumour development has been excluded (Isakov et al., 1981); the mechanisms underlying the LDV-mediated effects on tumour growth are unknown.

LDV induces complement (C')-dependent cytotoxic antibodies reactive with tumour surface antigen (TSA) of the Moloney murine sarcoma virus (Mo-MSV) non-producer (NP) transformant Sac (Weiland et al., 1987). Sac cells express a surface glycoprotein (46K antigen; Weiland & Thiel, 1985) that does not induce anti-46K antibodies when presented on the surface of live NP cells (Weiland et al., 1978). This cell surface antigen is probably encoded by one or more cellular genes, which are activated as a consequence of malignant transformation (Weiland & Thiel, 1985). Its relationship to the surface antigen that serves as a target for LDV-induced anti-TSA antibodies is unknown.

After LDV infection STU mice produce antibodies against their own Golgi antigen, as well as against TSA of Sac NP cells (Weiland et al., 1987). Both antibody specificities invariably appeared after LDV infection; they were used in a bioassay for LDV infection, which was as reliable as the conventional determination of LDH activity.

In order to elucidate the mechanism by which LDV influences tumour growth the anti-TSA immune response was further investigated. We compared the susceptibility of animals acutely and chronically infected with LDV to the syngeneic Mo-MSV NP transformant Sac.

Graded doses of Sac NP cells were transplanted into STU mice 1 day, 23 days and 5 months post-infection (p.i.) with LDV strain AGIA. Two weeks p.i. all LDVAGIA-infected mice possessed cytotoxic anti-Sac TSA antibodies, as evidenced in a C'-dependent antibody-mediated microcytotoxicity assay using 51Cr-labelled Sac cells (Weiland & Thiel, 1985). As can be seen from Table 1 transplantation of 10^4 Sac NP cells into mice infected 23 days earlier led to tumour development in only one of the six LDVAGIA-infected animals, whereas the five non-infected mice all developed tumours. Moreover, in the single tumour-positive LDVAGIA-infected mouse the latency period was prolonged. All six mice infected with LDVAGIA 23 days before tumour cell transplantation were protected against a challenge dose of 10^3 Sac NP cells, whereas all six non-infected animals developed tumours. Protection of mice infected 5 months before challenge was obvious.
Table 1. \(LDV_{AGIA}\) infection protects STU mice against syngeneic Sac tumours

<table>
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<tr>
<th>LDV(_{AGIA}) infection (days before tumour transplantation)</th>
<th>Numbers of transplanted Sac NP cells</th>
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<tr>
<td></td>
<td>(10^0)</td>
</tr>
<tr>
<td>Expt. 1 + (-23)*</td>
<td>5/5†</td>
</tr>
<tr>
<td></td>
<td>(5)‡</td>
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<tr>
<td>Expt. 2 + (-5 months)</td>
<td>5/5</td>
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<td></td>
<td>(6)</td>
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* All infected animals developed anti-Golgi and anti-Sac TSA antibodies.
† Number of mice with tumours/number of mice injected.
‡ Time in days after tumour transplantation at which a tumour was palpable.

Only after transplantation of 100 and 10 Sac NP cells. At this time the titre of the LDV-induced anti-TSA antibodies had decreased from 2100 to 300. No protection was observed when transplantation of as few as 10 Sac cells was performed 1 day after LDV\(_{AGIA}\) infection.

In addition to the LDV strains Row, Pla and AGIA (Weiland et al., 1987) the LDV strains 1 to 5 and C (Brinton et al., 1986) were also capable of inducing anti-TSA antibodies in infected STU mice. Independent of the virus dose all animals showing increased plasma LDH activity also possessed anti-TSA antibodies on day 7 p.i. (results not shown).

For subclass determination of the anti-Sac TSA antibodies in LDV-infected mice a \(^{125}\)I-labelled Protein A binding assay (Weiland & Thiel, 1985) was applied; they were demonstrable only in the IgM class (Fig. 1), independent of the time after LDV infection.

In contrast to STU or BALB/c mice AKR mice infected at the age of 2 or 6 months were negative for anti-TSA antibodies at 7 days p.i., independent of the virus dose. Most infected AKR mice exhibited low-titred anti-TSA antibodies (100) at days 12 to 20 p.i. However, BALB/c × AKR F\(_1\) hybrid mice produced high-titred antibodies (2100) as early as 7 days p.i.

To study the distribution of the TSA antigenic determinants on transformants of various origins hybridomas secreting antibodies against Sac TSA were prepared after fusion of SP2/0-Ag14 myeloma cells with spleen cells from a mouse 4 days after infection with LDV\(_{AGIA}\). Only one of about 800 hybridomas secreted the desired C\(^{'}\)-dependent cytotoxic anti-TSA antibodies and remained stable. Fusion experiments at later times p.i. did not increase the amount of anti-Sac TSA-secreting hybridomas; the only hybridoma isolated secreted monoclonal antibodies (MAbs) belonging to the IgM class. Ascitic anti-Sac TSA MAbs mediated cytotoxicity against Sac cells up to a dilution between \(10^{-5}\) and \(10^{-6}\). In an immunofluorescence assay on unfixed Sac cell monolayers coarse fluorescent granules were seen at the cell surface.

Lactoperoxidase-catalysed iodination of intact cells is used for identification of the molecules located at cell surfaces (Trowbridge et al., 1975). This procedure was successfully applied to identify a 46K Sac surface protein using MAbs of the IgG2a subclass (Weiland & Thiel, 1985). However, the LDV-induced anti-Sac TSA antibodies of the IgM class failed to precipitate any iodinated Sac surface antigens (results not shown).

To obtain information about the existence of a surface antigen on mouse tumour cells related to the TSA on Sac cells LDV-induced MAbs against Sac TSA were tested against several transformants of STU and BALB/c mouse origin, which included Mo-MSV transformants PV-TC-77, 112 (Weiland & Mussgay, 1982) and MSV85 C1 3 (Aaronson & Rowe, 1970), normal BALB/3T3 cells (CCL 163), simian virus 40-transformed BALB/3T3 cells (CCL 1631), 3-methylcholanthrene-transformed MethA cells of BALB origin (kindly provided by A. DeLeo, Sloan Kettering Cancer Center, New York, N.Y., U.S.A.) and L929 cells.

PV-TC-77, also expressing a 46K surface antigen like the Sac cells (Weiland & Thiel, 1985), bound LDV-induced anti-TSA antibodies as well as anti-46K antibodies (Fig. 2). None of the other transformants reacted with the anti-46K antibodies. The results were different with the LDV-induced anti-TSA MAbs. All cells of an L929 culture reacted with anti-TSA antibodies (Fig. 3), but the percentage of positive MethA or MSV85 C1 3 cells varied considerably (not shown). All three cell lines reacted in both the antibody-binding assay and the
C'-dependent antibody-mediated cytotoxicity assay. On the other hand, PV-TC-77 cells reacted only in the binding assay.

Although all nine LDV strains tested induced anti-TSA antibodies in STU mice, not all mouse strains were able to produce these antibodies after LDV infection (Weiland et al., 1987). AKR mice, for example, developed only a moderate and transient anti-TSA response, as shown in this study, and it is not known whether it allows tumour growth in AKR mice. Whether the capacity of LDV to induce anti-TSA antibodies reactive with tumours of different aetiology is indeed responsible for the established growth inhibition of certain tumours in chronically infected mice (Michaelides & Schlesinger, 1974) remains to be studied. So far the capacity of LDV to induce anti-TSA antibodies does not explain growth stimulation of certain tumours in acutely infected mice.

In contrast to the LDV-induced autoantibodies directed against antigens of the Golgi apparatus, which predominated as early as 7 days p.i. in the IgG subclasses 2a and 2b (Grossmann et al., 1989), anti-TSA antibodies were detectable exclusively in the IgM class during the whole observation period of more than 12 months. Many anti-Golgi apparatus antibody-producing hybridomas
could be detected between 5 and 21 days p.i. (Grossmann et al., 1989), but anti-TSA-producing hybridomas were only rarely found 4 to 12 days p.i.

Transformants differ from their progenitors by the pattern of their cell surface proteins, which are responsible for their invasive and proliferative properties (Hakomori, 1984). Our earlier studies have led to the detection of a glycosylated 46K (gp46) antigen on the surface of Sac cells that is not related to known C-type virus structural antigens (Weiland & Thiel, 1985) and here we studied whether this antigen might be the target of LDV-induced anti-TSA antibodies. In contrast to anti-gp46, both monoclonal and polyclonal LDV-induced anti-TSA antibodies failed to precipitate surface antigen(s) of Sac cells (probably because of their IgM nature). Competitive binding studies indicated that three anti-gp46 MAbs did not block the reactivity of LDV-induced TSA MAbs in a microcytotoxicity assay (results not shown). Since anti-TSA antibodies reacted with transformants such as MethA, MSV85 C1 3 and L929, which are negative for gp46 surface expression (data not shown), we conclude that gp46 and TSA are antigenically unrelated.

The mechanisms leading to induction of anti-TSA antibodies by live LDV, but not by high doses of inactivated virus (data not shown), are unknown. Further studies will show whether TSA expression is indeed the prerequisite of LDV-mediated tumour inhibition by LDV-induced anti-TSA antibodies.

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References


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