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Enhanced tumor susceptibility of immunocompetent mice infected with lymphocytic choriomeningitis virus*

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Summary. Mice infected i.v. with high doses of lymphocytic choriomeningitis virus (LCMV; 10^5–10^6 plaque-forming units) 8–10 days prior to challenge with the methylcholanthrene-induced fibrosarcoma tumor cell line MC57G or the melanoma cell line B16 tumor cells showed an enhanced tumor susceptibility with respect to both growth kinetics of the tumor and the minimal dose necessary for tumor take. After transient initial growth, MC57G tumor cells were all rejected by uninfected C57BL/6 mice by day 14. Mice preinfected i.v. with LCMV 3 weeks before or at the time of tumor challenge, but not those infected 2 months before or 7 days after, showed increasing tumor growth, the tumor take being 100% for 10^6, 50% for 10^5 and 37% for 10^4 MC57G tumor cells injected into the footpad compared with resistance to 10^6 cells in normal mice. B16 melanoma cells also grew more rapidly in LCMV-preinfected mice and by day 40 tumors were established with about 100 times fewer cells, i.e. about 10^3 compared with 3×10^4–3×10^5 for uninfected mice. Analysis of the growth of tumor cells in normal and in LCMV-carrier mice revealed that the latter mice were not more susceptible to LCMV-infected than to uninfected MC57G. Since LCMV-carrier mice fail to mount LCMV-specific T cell responses, these results suggest that anti-LCMV-specific T cells may be responsible for acquired immunodeficiency hampering immune surveillance against the tumors studied.

Introduction

There are several examples where an immune response against a tumor was enhanced by introducing strong foreign antigens either by chemical modification [3, 16], virus infection [5, 19, 30, 35–37, 52] or mutagenesis of cellular antigens [48]. In an attempt to induce virus-mediated heterogenization or xenogenization of tumor cells, we infected tumor cells with lymphocytic choriomeningitis virus (LCMV) to enhance resistance of mice against the tumor [7, 20, 22, 36]. Unexpectedly, infected tumor cells or subsequent tumor cell challenges into preinfected mice were not only *not* rejected but, in contrast, progressed more efficiently and more rapidly when compared to uninfected tumor cells in normal mice. Such enhancement of growth of LCMV-infected tumors has been seen before [20, 47, 53]. It also has been known for some time that mice infected with certain isolates of LCMV exhibit a more or less severe immunodeficiency [16, 20, 23, 27, 34, 42] that depends upon a variety of parameters, such as virus dose, virus isolate, mouse strain and time of infection [27, 42]. Immune suppression mediated by LCMV-WE is probably also responsible for both the failure of mice infected with high doses of virus to clear virus rapidly and their succumbing to choriomeningitis [7, 20, 22, 46]. This acquired immune suppression is probably not caused by the virus itself, since LCMV-carrier mice are known to respond immunologically within normal ranges [7, 20, 22, 23, 27, 38]. Recent studies have revealed that anti-LCMV-specific cytotoxic T cells appear to be responsible for the immune suppression; their elimination by treatment with antibodies against $CD8^+$ T cells prevents immune suppression and immune suppression can be adoptively transferred by LCMV-specific cytotoxic T cells [27]. In the present study we analyzed the basic parameters of acquired, LCMV-triggered impairment of immune surveillance against tumors. This model situation may offer some insight into the pathogenesis of tumors in patients with acquired immunodeficiency syndrome [8, 12, 18, 29, 40, 41, 43].

Materials and methods

Mice. Male C57BL/6 mice, 6–12-weeks old were obtained from the breeding colony of the Institut für Zuchthygiene, Tierspital Zürich, Switzerland.

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Virus. The LCMV-WE strain [7, 20, 22] was originally obtained from Prof. F. Lehmann-Grube, Hamburg, FRG. Virus stocks were grown on L-929 cells and reached an approximate titer of 5×10^6 plaque-forming units (pfu)/ml. The titer was determined by plaque formation on L cells [24, 25]; one mean infectious dose for 50% (ID_{50}) of the animals corresponded to about 0.3–0.1 pfu [25]. Virus dilutions were made in minimal essential medium (MEM) containing 1%–5% heat-inactivated fetal calf serum (FCS). LCMV-carrier mice were induced by injecting newborn C57BL/6 mice during the first 18 h after birth with 10^5 pfu LCMV-WE i.p. [20, 22]. Their carrier status was monitored 6 weeks after birth and at the time of tumor challenge.

Tumor cells and their infection with LCMV. Methylcholanthrene-induced MC57G cells ($H\text{-}2^b$) [1] had been obtained originally from Dr. B. B. Knowles, Wistar Institute, Philadelphia. Melanoma B16 cells [14, 15] had been obtained from Dr. G. Klein, Karolinska Institute, Stockholm. These cells were cultured in MEM (5% FCS) at 37° C and 5% CO_2. Stocks of both tumor cell lines were prepared after one or two in vivo passages with subsequent in vitro culture. The cell culture stocks were monitored for mycoplasma and were found to be negative. Both tumor cell lines were readily infected by LCMV-WE by adding 10^3 pfu LCMV-WE to subconfluent cell monolayers in 75-cm^2 Falcon flasks for 48 h. More than 95% of the cells were infected, as estimated by immunofluorescence [27]. Cell dilutions for injection were prepared in MEM without FCS. Cells were inoculated into the foodpad (i.f.) in a total volume of 30 µl.

Antibodies. The rat IgG 2b, monoclonal antibodies YTS 169.4.2 (anti-CD8) and YTS 191.1.2 (anti-CD4) were a gift of Dr. H. Waldmann, Cambridge, and were prepared as described by Cobbold et al. [9]. The ascitic fluids from tumor-bearing pristane-primed (LOU × DA)F1 rats were partially purified by precipitation with 50% $(NH_4)_2SO_4$. The precipitates were redissolved in phosphate-buffered saline (PBS) to yield protein concentrations of about 15 mg/ml containing 2–3 mg active antibodies.

T cell subset depletion. Thymectomized mice were given two intravenous (i.v.) injections of monoclonal antibodies YTS 169.4.2 or YTS 191.1.2 (approximately 1 mg active antibody) 2–4 days apart.

Monitoring of T cell subsets. Efficiency and specifity of the depletion treatment were monitored by an immunofluorescence assay [9]. Air-dried blood smears were fixed on glass microscope slides with acetone and ethanol. Before staining, the smears were washed in PBS containing 1% bovine serum albumin. Either anti-CD4 or anti-CD8 antibody was added to each slide at a concentration of 0.02 mg/ml in PBS containing 1% bovine serum albumin. After incubation and washing with PBS, fluorescein-isothiocyanate-labeled mouse anti-(rat IgG 2b) antibody was added at a concentration of 0.01 mg/ml in PBS containing 1% bovine serum albumin and 1 mg/ml propidium iodide. After additional washing steps, slides were mounted with glass coverslips using 90% glycerol, 10% PBS, and 1 mg/ml *p*-phenylenediamine and monitored in a fluorescence microscope. Depletion was >95%.

Measurement of tumor growth. The thickness of footpads was measured with a spring-loaded caliper (Kröplin, Schluchtern, Hessen, FRG) after injection of tumor cells every day or every second day. In experiments with B16 melanoma cells, the occurrence of a black spot in the footpad was also registered. Preliminary experiments had shown that tumors that caused footpad thickness greater than 5 mm were not subsequently rejected. Mice were killed when feet with tumors had reached a diameter of 7–8 mm at a time when they still were comfortable.

Results

Normal mice reject LCMV-WE-infected tumor cells less efficiently than uninfected tumor cells

In an attempt to heterogenize tumor cells by infection with LCMV to improve their rejection by the host, the following growth patterns were observed in normal C57BL/6 mice or in mice preimmunized with a low (10^3 pfu) dose of LCMV 4 weeks before tumor cell challenge (Fig. 1). Except for a very early transient swelling (on day 3) immune mice showed no tumor growth for up to 50 days when 3×10^5 LCMV-infected tumor cells were injected into the footpad; normal mice challenged with 3×10^5 infected tumor cells exhibited tumor growth. Only a transient variable tumor growth was seen with 3×10^5 normal (uninfected) MC57G cells between days 3 and 12; 3×10^6 cells grew in both LCMV-immune and normal mice. Thus infected tumor cells exhibited enhanced rather than reduced growth in normal hosts. This result suggested that the high dose of LCMV released by infected tumor cells may cause an immune suppression [23, 34, 51] enhancing tumor growth.

The swelling reactions observed in normal mice inoculated with uninfected tumor cells usually exhibited two phases dependent upon the dose of cells inoculated. In the first phase, tumor cells may grow to some measurable or not measurable level up to days 8–12 then they are rejected completely (e.g. Fig. 1, top panel left, or Fig. 2, first panel); alternatively they continue to grow (Fig. 1, bottom panel left). The footpad swelling in normal mice, which was caused by growth of infected tumor cells, was a composite of two distinct swelling reactions: the first swelling was caused mainly by LCMV-specific T cells mediating a delayed-type swelling reaction; the subsequent swelling was due to tumor growth [7, 20, 22]. Therefore tumor growth was assessed usually after day 12. Mice were killed when the footpads reached a thickness of 70–80 mm.

To demonstrate that enhanced tumor growth was caused by effects of systemic LCMV infection in mice and was independent of tumor cells being infected themselves with LCMV, the following experiments were performed. Tumor cells were titrated in uninfected C57BL6 mice and in mice infected 8 days previously with a high dose of LCMV-WE (10^6 pfu) i.v. The latter mice exhibited a drastically increased susceptibility to tumor growth (Table 1): normal mice rejected 10^6 uninfected MC57G cells, whereas three of eight infected mice exhibited tumor growth after injection of 10^4 tumor cells by day 30.

Time dependence of tumor growth enhancement and LCMV infection

The time dependence of the effect of LCMV infection on growth of normal tumor cells was assayed in the following experiments: mice were infected with 10^3 or 10^6 pfu LCMV-WE i.v. and were challenged with 10^5 MC57G cells into the footpads (Fig. 2). Preinjection with low or high doses of LCMV-WE i.v. enhanced the growth of uninfected tumor cells if the virus was injected 8 days before or on the same day as the tumor cells, but not if it was injected on day 7 after tumor cell challenge. Low doses of LCMV enhanced tumor growth if injected on day −8 but less consistently so if injected on day 0. If mice had been infected with LCMV 50 days previously, uninfected tumor cells grew rapidly in mice preinfected with high doses of LCMV (10^6 pfu, apparently still causing some immune suppression) but were rejected in mice immunized

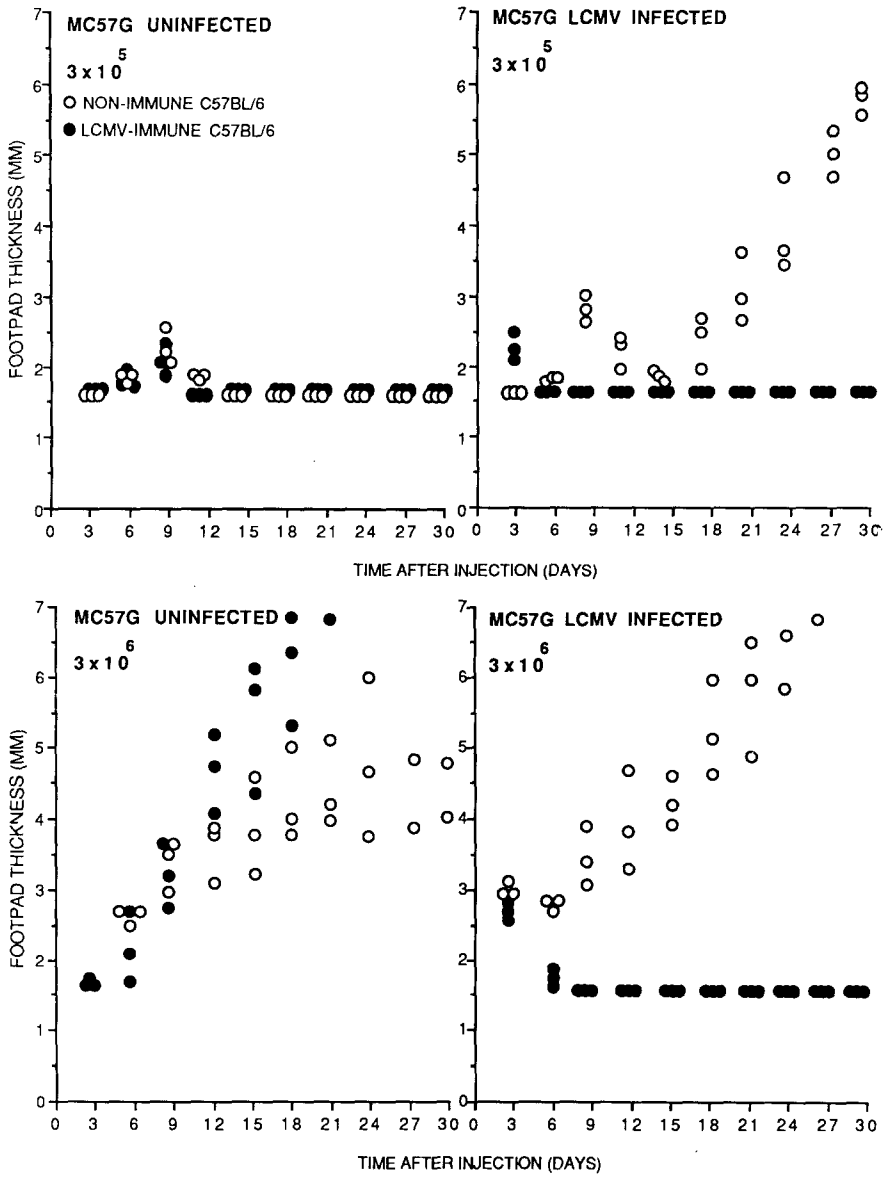

Fig. 1. Tumor growth in normal or LCMV-immune mice: C57BL/6 mice were immunized with lymphocytic choriomeningitis virus (LCMV; –28 days, 2×10^3 pfu i.v., ●) and normal mice (○) were challenged on day 0 with uninfected or LCMV-infected MC57G (either 3×10^5 or 3×10^6 cells) into the right footpad only. Three individual mice were tested per group at each time point. Mice with tumors causing feet to swell to more than 70 mm were killed

with a lower dose of 10^3 pfu LCMV. Thus the tumor-growth-enhancing effects of LCMV were systemic and dependent on both time and dose.

Titration of the increase of susceptibility to MC57G or of melanoma B16 tumor cells

Susceptibility to MC57G was about 100 times increased in LCMV-preinfected mice. Since resistance to uninfected MC57G in normal C57BL/6 mice was usually very pronounced, this titration of an effective tumor cell dose did not reveal a greater factor of enhancement. To evaluate whether LCMV-induced enhancement of tumor growth was specific for MC57G, growth enhancement of susceptibility to the melanoma B16 cell was assessed with the same protocols; comparable results were obtained. LCMV-immune mice efficiently rejected 10^5 LCMV-infected B16 tumor cells whereas normal mice failed to do so (data not shown). Preinfection of mice with LCMV enhanced B16 tumor growth with respect to the time of appearance of black tumors in the footpad, overall growth of the tumor and tumor takes by day 37. Whereas normal mice injected with 3×10^3 B16 cells showed no tumor on day 37, LCMV(10^6 pfu i.v.)-preinfected mice exhibited visible tumor growth in three out of six injected footpads (Fig. 3).

Effect of depletion of T cell subsets on resistance to tumor growth in normal or LCMV-preinfected mice

Studies on the immunosuppressive action of LCMV infection on antibody responses in mice had suggested that virus-specific cytotoxic T cells were instrumental in inducing the acquired immunodeficiency [27]. Therefore the effect of depletion of cytotoxic T cells on the observed tumor growth enhancement caused by LCMV was evaluated (Fig. 4). Thymectomized C57BL/6 mice were infected with 1×10^6 LCMV-WE i.v. on day –8. A second test group was not pretreated with virus. On days –9 and –7 two groups of mice were treated either with anti-CD4 or anti-CD8 monoclonal antibodies. These treatments had pre-

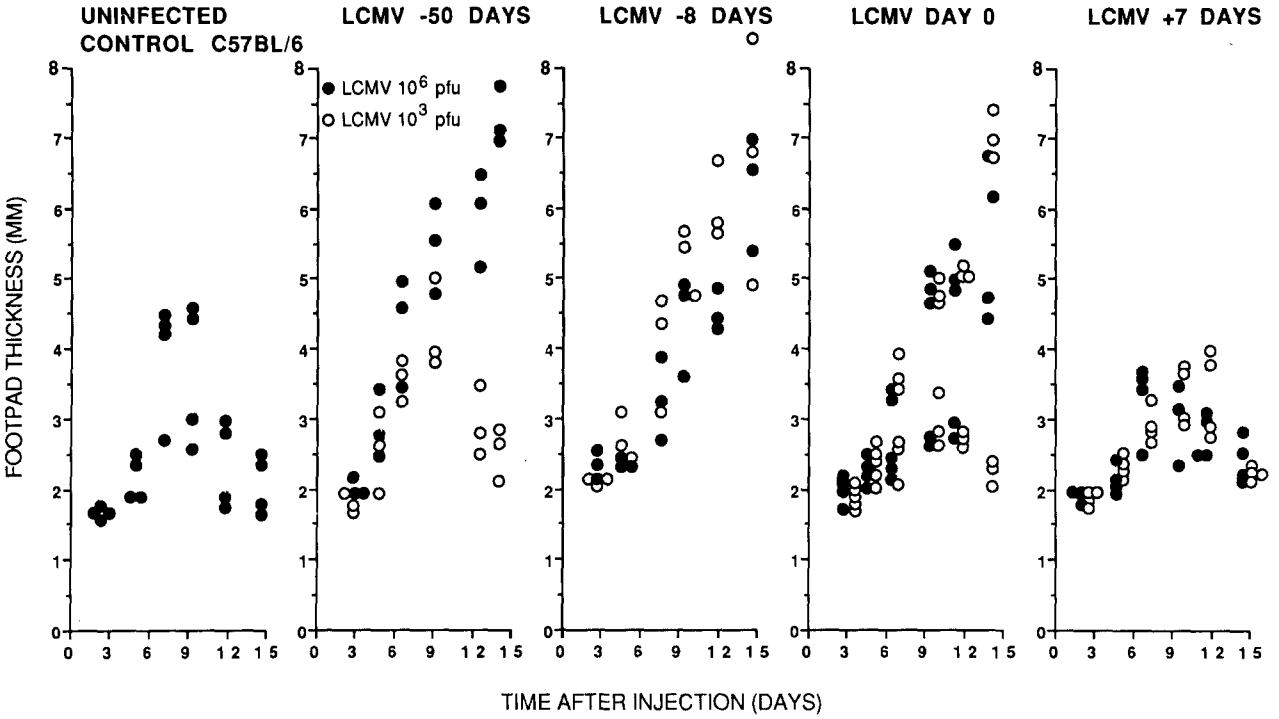

Fig. 2. Time and dose dependence of tumor growth in footpads: facilitating effects of LCMV infection (10^6 pfu i. v. or 10^3 pfu i. v.) on tumor growth of 10^5 MC57G cells injected into footpads. Measurements of individual feet are shown

Table 1. Titration of effective MC57G tumor cell dose in normal and LCMV-preinfected (10^6 pfu i. v. on day –8) C57BL/6 mice

Dose of MC57G	Numbers of feet with tumor growth/numbers of feet injected: time after tumor cell injection					
	Day 12		Day 15		Day 30	
	Uninfected mice	LCMV-infected mice	Uninfected mice	LCMV-infected mice	Uninfected mice	LCMV-infected mice
10^6	8/8	8/8	0/8	8/8	0/8	8/8
10^5	5/8	8/8	0/8	7/8	0/8	4/8
10^4	4/8	4/8	0/8	3/8	0/8	3/8
10^3	0/8	0/8	0/8	0/8	0/8	0/8

viously been shown to deplete thymectomized mice of either helper T cells or cytotoxic T cells [26]. The control group was not treated. On day 0 all mice were inoculated with 3×10^5 MC57G cells into the footpad. Uninfected animals that were not treated with antibody were able to reject the growing tumor (Fig. 4). Normal animals that had been treated with either anti-CD4 or anti-CD8 antibodies were not able to reject the MC57G tumor, although tumors grew more slowly than in LCMV-pretreated mice (Fig. 4, left part). Thus, both $CD4^+$ and $CD8^+$ T cells seemed to be involved somehow in immune surveillance of the tumor studied. The same treatments of LCMV-pretreated mice (1×10^6 pfu, day –8) had no effects on tumor growth. Thus, depletion of cytotoxic T cells, including anti-LCMV cytotoxic T cells, did not drastically reduce the tumor-growth-enhancing effects of LCMV MC57G. Unfortunately these effects cannot be judged properly because of the direct effects of depletion of T cell subsets on rejection of tumor cells.

Tumor resistance in nude mice

The experiments presented so far suggested that general immune surveillance was impaired in immunocompetent mice preinfected with LCMV. In a previous series of experiments we had shown that LCMV-triggered immunosuppression, assessed by reduced antibody response to a T-cell-independent antigen, depended upon virus-specific T cells [27]; in those studies thymus and T-cell-deficient nu/nu mice infected with LCMV exhibited normal IgM respones. Therefore the influence of LCMV infection on tumor growth was assessed in nu/nu mice (Fig. 5). LCMV-infected MC57G exhibited slower tumor growth for MC57G doses of 10^5 or 10^4 when compared to uninfected tumor cells in nu/nu mice, probably signalling natural killer (NK) effects [21, 35, 50]. However, overall, nu/nu mice were considerably more susceptible to MC57G (and to B16 as well, data not shown) as indicated by the fact that all mice showed tumor growth when injected with 10^4 tumor cells irrespective of whether or not they were infect-

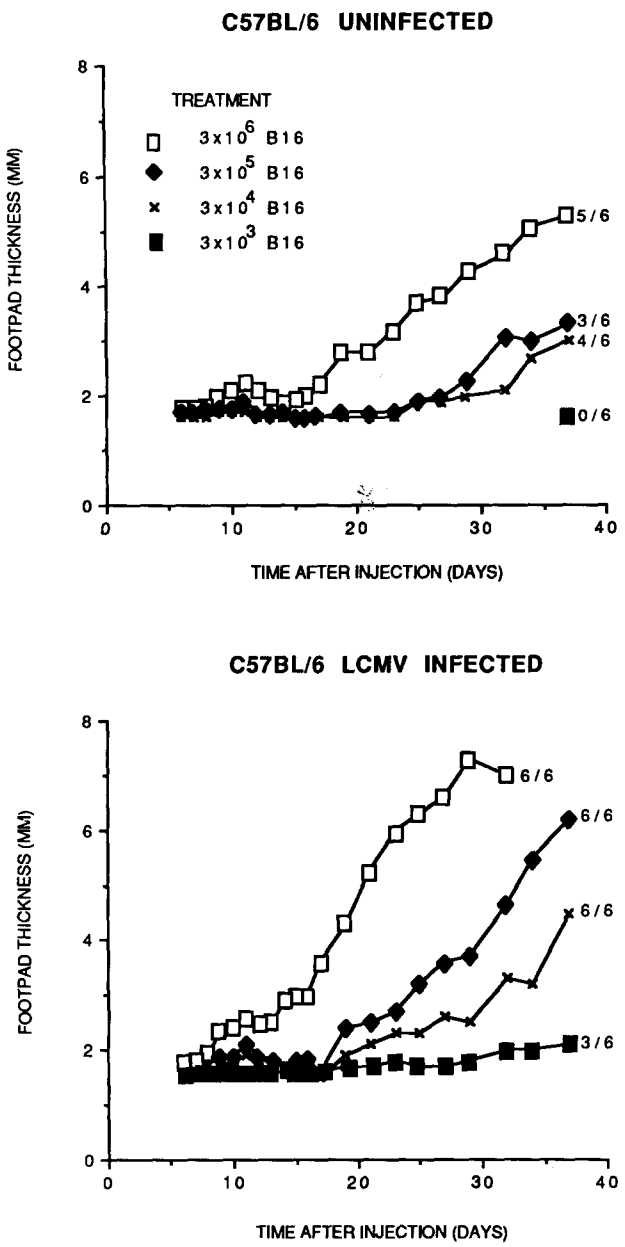

Fig. 3. Growth of melanoma B16 in footpads of uninfected or LCMV-infected C57BL/6 mice: mice were infected with LCMV (10^6 pfu i.v.) 8 days prior to challenge with B16 cells into footpads. Numbers indicate number of visible tumors per number of feet injected. The results represent means of six individual footpads, SEM were below 0.5 mm for all measurements below 4 mm thickness, and below 1.0 mm for values >4 mm thickness

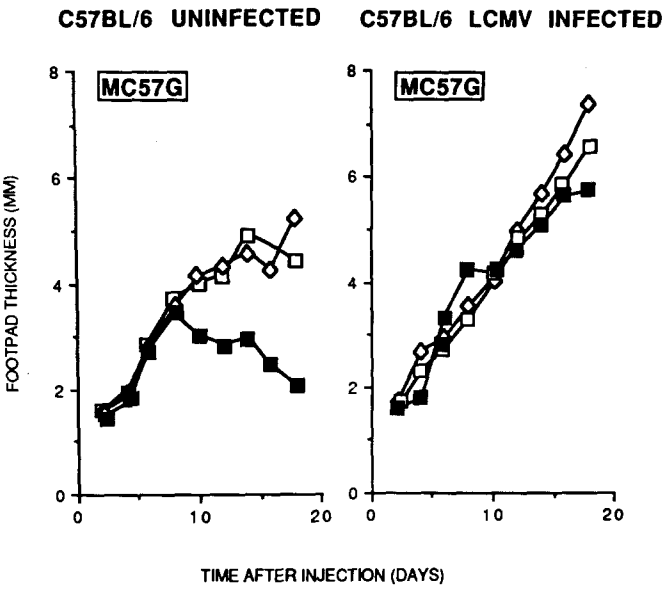

Fig. 4. Effects of depletion in vivo of T cells subsets on tumor growth: thymectomized C57BL/6 mice uninfected or LCMV-infected (–8 days, 1×10^6 pfu) were pretreated or not (■) with anti-CD4 (◇) or anti-CD8 (□) mAb (day –9, day –7) and challenged on day 0 with 3×10^5 tumor cells. Data represent means of six footpads; SEM were <0.5 mm for thickness measurements <4 mm, and <1.0 mm for those >4 mm

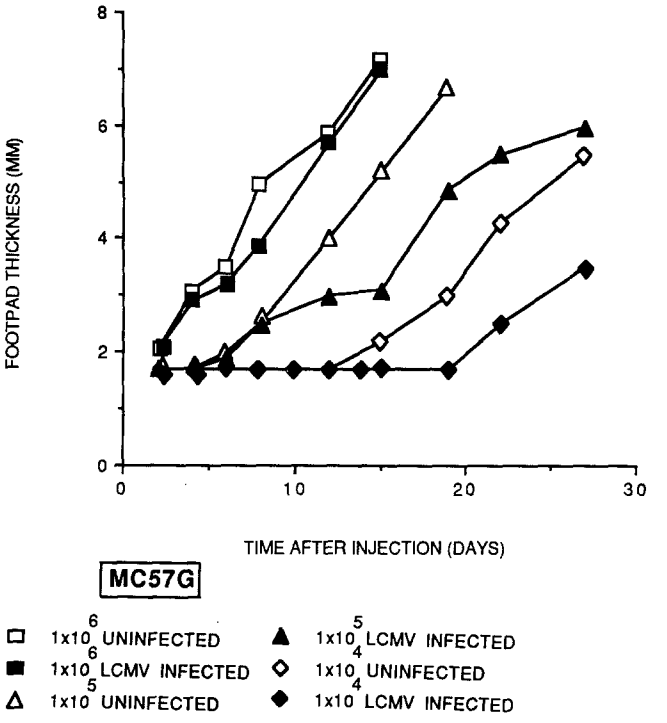

Fig. 5. Growth of MC57G in nude mice: LCMV-infected or uninfected tumor cells were injected into footpads of uninfected C57BL/6 nu/nu mice. Means of six to eight footpads, SEM were smaller than 0.5 mm for values <4 mm thickness and smaller than 1.2 mm for the other values

ed. Thus both NK-like and T-cell-dependent mechanisms were involved in immune surveillance against the tumors studied. Since LCMV-induced NK-like resistance suppressed tumor growth, the results obtained in the previous experiments suggested that NK activities may be suppressed in LCMV-infected normal mice.

Unimpaired immune surveillance in LCMV-carrier mice

To evaluate whether LCMV itself or rather the immune response to LCMV was responsible for impairment of immune surveillance, the following experiments were performed. Neonatally infected LCMV-carrier mice [20, 22, 46] and uninfected normal C57BL/6 mice were injected with LCMV-infected or uninfected MC57G cells. Tumor growth kinetics were monitored as shown in Fig. 6. LCMV-infected MC57G grew in LCMV-carrier mice

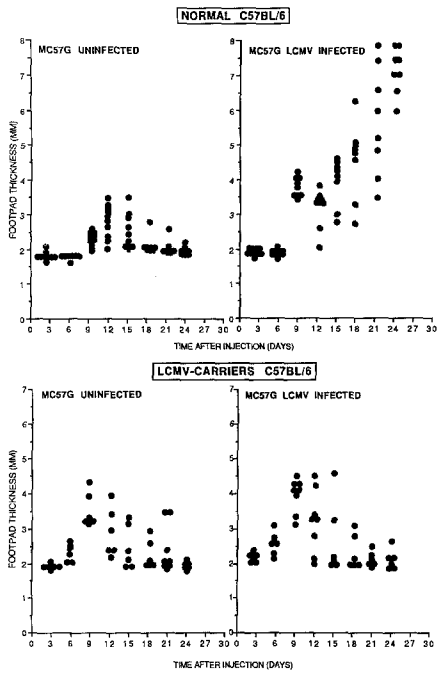

Fig. 6. Tolerance to LCMV prevents enhancement of tumor growth by LCMV: 3×10^5 uninfected or LCMV-infected MC57G cells were injected into footpads of normal C57BL/6 or of neonatally infected LCMV-carrier mice. Eight individual feet were measured and are shown

comparably to uninfected MC57G in either normal or carrier mice. Therefore the presence of LCMV in carrier mice did not impair immune surveillance in contrast to the response in normal mice undergoing an acute LCMV infection or recovering from it.

Discussion

Mice acutely infected with LCMV-WE were, depending upon the virus dose, more susceptible to growth of a fibrosarcoma (MC57G) and a melanoma (B16) tumor cell line for some weeks after the infection. This enhanced susceptibility was not caused by the poorly lytic virus itself, since LCMV-carrier mice that are immunologically tolerant to LCMV were not more susceptible than uninfected mice. Also, thymus-deficient nude mice infected with LCMV are not more susceptible than uninfected nude control mice to tumor growth; in these mice LCMV infection seemed to increase resistance to MC57G (but also to B16; data not shown) slightly, most probably because of NK activity [21, 28, 35, 50]. Treatment of infected T-cell-competent mice with anti-CD4 or anti-CD8 antibodies could not reveal a T cell mechanism triggered by LCMV infection that could be made responsible for the enhancement of tumor growth, because such treatments impaired tumor cell rejection also in uninfected mice. Nevertheless, together with earlier experiments demonstrating that LCMV-specific cytotoxic T cells were involved in LCMV-triggered immune suppression, the data presented are compatible with the view that T cell immunity and immunopathology to LCMV cause an acquired immunodeficiency resulting an apparently generalized impairment of the immune surveillance mechanism, causing enhanced tumor growth.

This model situation in mice recalls the enhanced Kaposi sarcoma tumor incidence in patients infected with human immunodeficiency virus (HIV) [8, 12, 18, 29, 40, 41, 43]. HIV also causes an acquired immunodeficiency syndrome, though of considerably more chronic development than that induced by LCMV. In both examples, immune surveillance is apparently reduced and in both cases there is direct (for LCMV) or indirect evidence that the impairment of immune surveillance may be, at least in part, a result of immunopathological mechanisms rather than direct lytic effects of the virus (reviewed in [12, 44, 49, 55, 56]). The comparison of immunologically T-cell-tolerant LCMV-carrier mice with acutely infected normal mice illustrates the point most clearly that presence of LCMV alone is not sufficient to cause enhanced tumor growth. This notion may be supported by the results in nude mice, suggesting that LCMV-triggered and probably interferon-triggered NK cell activity may actually slow tumor growth. These data, therefore, may indicate that NK-like mechanisms are impaired by LCMV-induced immunopathology. Alternatively these results suggest that the detectable suppressive effect on specific immune surveillance may actually be underestimated if NK effector mechanisms are operational.

This study cannot define the effector cells or mechanisms impaired by the immune response of mice against LCMV. Since both $CD4^+$ and $CD8^+$ T cells are apparently somehow involved in control of growth of MC57G [4, 13, 17, 45, 54], it was not possible to define the pathogenesis of the observed enhancement in greater detail. Previous experiments on LCMV-induced immune suppression of antibody responses using comparable experimental conditions [27, 42], however, have revealed that LCMV-immune $CD8^+$ T cells seem to be involved in causing immune suppression. This mechanism may therefore also apply here, since most of the acute immunopathological effects of LCMV studied so far have been shown to be caused by antiviral $CD8^+$ T cells [7, 10, 11, 20, 22]. Although the direct target of this T-cell-mediated immunopathology is not known, there are various possible candidates. Several members of the lymphohemopoietic cell pool, including macrophages, dendritic cells [31–33], T helper ($CD4^+$) cells [2, 39], NK cells and possibly B cells, may be infected with LCMV and may, therefore, be destroyed by anti-LCMV $CD8^+$ T cells. Reduction in any of these cell compartments, in particular NK cells, macrophages and dendritic/antigen-presenting cells, may hamper immune surveillance against tumors by T cells, NK cells, macrophages and others, including interleukin-dependent mechanisms, directly or indirectly [4, 13, 17, 45, 54].

References

1. Aden DP, Knowles BB (1976) Cell surface antigen coded for the human chromosome 7. Immunogenetics 3: 209
2. Ahmed R, King C, Oldstone MBA (1987) Virus-lymphocyte interaction: T cells of the helper subset are infected with lymphocytic choriomeningitis virus during persistent infection in vivo. J Virol 61: 1571

3. Bekesi JG, St-Arneault G, Holland JF (1972) Immunogenicity of leukemia L1210 cells after neuraminidase treatment. J Natl Cancer Inst 49: 107
4. Boog CJP, Boes J, Melief CJM (1988) Stimulation with dendritic cells decreases or obviates the CD4+ helper cell requirement in cytotoxic T lymphocyte responses. Eur J Immunol 18: 219
5. Boone CW, Blackman K (1972) Augmented immunogenicity of tumor cell homogenates infected with influenza virus. Cancer Res 32: 1018
6. Bro-Jörgensen K, Volkert M (1974) Defect in the immune system of mice infected with lymphocytic choriomeningitis virus. Infect Immun 9: 605
7. Buchmeier MJ, Welsh RM, Dutko FJ, Oldstone MBA (1980) The virology and immunobiology of lymphocytic choriomeningitis virus infection. Adv Immunol 30: 275
8. Centers of Disease Control Task Force (1982) Epidemiological aspects of the current outbreak of Kaposi's sarcoma and opportunistic infections. N Engl J Med 306: 248
9. Cobbold SP, Jayasuriya A, Nash A, Prospero TD, Waldmann H (1984) Therapy with monoclonal antibodies by elimination of T cell subsets in vivo. Nature 312: 548
10. Cole GA, Nathanson N, Prendergast RA (1972) Requirement for thetabearing cells in lymphocytic choriomeningitis virus-induced central nervous system disease. Nature 238: 335
11. Doherty PC, Zinkernagel RM (1974) T cell-mediated immunopathology in viral infection. Transplant Rev 19: 89
12. Fauci AS (1988) The human immunodeficiency virus: Infectivity and mechanisms of pathogenesis. Science 239: 617
13. Fernandez-Cruz E, Woda BA, Feldman JD (1980) Elimination of syngeneic sarcomas in rats by a subset of T lymphocytes J Exp Med 152: 823
14. Fidler IJ (1973) Selection of successive tumor lines for metastasis. Nature 242: 148
15. Fidler IJ, Nicolson GL (1976) Brief communication: organ selectivity for implantation survival and growth of B16 melanoma variant tumor lines. J Natl Cancer Inst 57: 1199
16. Fujiwara H, Moriyama Y, Suda T, Tsuchida T, Shearer GM, Hamaoka T (1984) Enhanced TNP-reactive helper T cell activity and its utilization in the induction of amplified tumor immunity that results in tumor regression. J Immunol 132: 1571
17. Fujiwara H, Yoshioka T, Shima J, Kosugi A, Itoh K, Hamaoka T (1986) Helper T cells against tumor-associated antigens (TAA): preferential induction of helper T cell activities involved in anti-TAA cytotoxic T lymphocyte and antibody responses. J Immunol 136: 2715
18. Gold K, Thomas D, Garret TJ (1982) Aggressive Kaposi's sarcoma in a heterosexual drug addict (New York City). N Engl J Med 307: 498
19. Hamburg V, Svet-Moldavsky G (1967) Suppression of viral and chemical carcinogenesis by means of artificial heterogenization. Nature 215: 230
20. Hotchin J (1971) Persistent and slow virus infections. Monogr Virol 3: 1
21. Jacobson S, Friedman RM, Pfau CJ (1981) Interferon induction by lymphocytic choriomeningitis viruses correlates with maximum virulence. J Gen Virol 57: 275
22. Lehmann-Grube F (1971) Lymphocytic choriomeningitis virus. Virol Monogr 10: 1
23. Lehmann-Grube F (1984) Bacterial and viral inhibition and modulation of host defences. Academic Press, London, pp 211–242
24. Lehmann-Grube F, Ambrassat J (1977) A new method to detect lymphocytic choriomeningitis virus-specific antibody in human sera. J Gen Virol 37: 85
25. Lehmann-Grube F, Assmann-Wischer U, Löliger C, Moskophidis D, Löhler J (1985) Mechanism of recovery from acute virus infection. I. Role of T lymphocytes in clearance of lymphocytic choriomeningitis virus from spleens of mice. J Immunol 134: 608
26. Leist TP, Cobbold SP, Waldmann H, Aguet M, Zinkernagel RM (1987) Functional analysis of T lymphocyte subsets in antiviral host defense, J Immunol 138: 2278
27. Leist TP, Eppler M, Rüedi E, Zinkernagel RM (1988) Virus triggered AIDS in mice is a T cell mediated immunopathology caused by virus-specific cytotoxic T cells: prevention by tolerance or by treatment with anti-CD8 antibodies. J Exp Med 167: 1749
28. Leist TP, Eppler M, Zinkernagel RM (1989) Enhanced virus replication and inhibition of lymphocytic choriomeningitis virus disease in anti-IFN gamma-treated mice. J Virol 63: 2813
29. Levy JA, Ziegler JL (1983) Acquired immune deficiency syndrome (AIDS) is an opportunistic infection and Kaposi's sarcoma results from secondary immune stimulation. Lancet 2: 78
30 Lindenmann J, Klein PA (1967) Viral oncolysis: increased immunogenicity of host cell antigen associated with influenza virus. J Exp Med 126: 93
31. Löhler J, Lehmann-Grube F (1981) Immunopathologic alterations of lymphatic tissues of mice infected with lymphocytic choriomeningitis virus. I Histopathologic findings. Lab Invest 44: 193
32. Mims CA (1966) Immunofluorescene study of the carrier state and mechanism of vertical transmission in lymphocytic choriomeningitis virus infection in mice. Pathol Bacteriol 91: 395
33. Mims CA, Subrahmanyan TP (1966) Immunofluorescence study of the mechanism of resistance to superinfection in mice carrying the lymphocytic choriomeningitis virus. J Pathol Bacteriol 91: 403
34. Mims CA, Wainwright S (1968) The immunodepressive action of lymphocytic choriomeningitis virus in mice. J Immunol 101: 717
35. Minato N, Bloom BR, Jones Ch, Holland J, Reid LM (1979) Mechanism of rejection of virus persistently infected tumor cells by athymic nude mice. J Exp Med 119: 1117
36. Molomut N, Padnos M, Gross L, Satory V (1964) Inhibition of a transplantable murine leukaemia by a lymphocytopenic virus. Nature 204: 1003
37. Moore A (1960) The oncolytic viruses. Prog Exp Tumor Res 1: 411
38. Oldstone MBA, Tishon A, Chiller JM, Weigle WO, Dixon FJ (1973) Effect of chronic viral infection on the immune system: I. comparison of the immune responsiveness of mice chronically infected with LCM virus with that of noninfected mice. J Immunol 110: 1268
39. Popescu M, Löhler J, Lehmann-Grube F (1979) Infectious lymphocytes in lymphocytic choriomeningitis virus carrier mice. J Gen Virol 42: 481
40. Quinn TC, Mann JM, Curran JW, Piot P (1986) AIDS in africa: an epidemiologic paradigm. Science 234: 955
41. Rogers MF, Morens DM, Stewart JA et al. (1983) National casecontrol study of Kaposi's sarcoma and pneumocystis carinii pneumonia in homosexual men: II. Laboratory results. Ann Intern Med 99: 151
42. Roost HP, Charan S, Gobet R, Rüedi E, Hengartner H, Althage A, Zinkernagel RM (1988) An acquired immunodeficiency in mice caused by infection with lymphocytic choriomeningitis virus. Eur J Immunol 18: 511
43. Schroff R, Gottlieb MS, Prince HE et al. (1983) Immunological studies of homosexual men with immunodeficiency and Kaposi's sarcoma. Clin Immunol Immunopathol 27: 300
44. Shearer GM (1986) AIDS: an autoimmune pathologic model for the destruction of a subset of helper T lymphocytes. Mount Sinai 53: 609
45. Takai Y, Kosugi A, Yoshioka T, Tomita S, Fujiwara H, Hamaoka T (1985) T-T cell interaction in the induction of delayed-type hypersensitivity (DTH) respones: vaccinia virus-reactive helper T cell activity involved in enhanced in vivo induction of DTH responses and its application to augmentation of tumor-specific DTH responses. J Immunol 134: 108
46. Traub E (1936) Persistence of lymphocytic choriomeningitis virus in immune animals and its relation to immunity. J Exp Med 63: 847
47. Traub E (1962) Can LCMV virus cause lymphomatosis in mice? Arch Gesamte Virusforsch 11: 667
48. Van Pel A, Boon Th (1982) Protection against a nonimmunogenic mouse leukemia by an immunogenic variant obtained by mutagenesis. Proc Natl Acad Sci USA 79: 4718
49. Walker CM, Moody DJ, Stites DP, Levy JA (1986) CD8+ lymphocytes can control HIV infection in vitro by suppressing virus replication. Science 234: 1563
50. Welsh RM, Zinkernagel RM (1977) Heterospecific cytotoxic cells during acute lymphocytic choriomeningitis virus infection. Nature 268: 646
51. Wheelock EF, Toy ST (1973) Participation of lymphocytes in viral infections. Adv Immunol 16: 123

52. Wise KS (1977) Vesicular stomatitis virus-infected L1210 murine leukemia cells: increased immunogenicity and altered surface antigens. J Natl Cancer Inst 58: 83
53. Youn JK, Barski G (1966) Interference between lymphocytic choriomeningitis and rauscher leukemia in mice. J Natl Cancer Inst 37: 381
54. Yu AS, Bernstein ID (1982) T helper cells in immune mice amplify the primary anti-tumor cytotoxic response. J Immunol 129: 856
55. Zeigler JL, Stites DP (1986) Hypothesis: AIDS is an autoimmune disease directed at the immune system and triggered by a lymphotropic retrovirus. Clin Immunol Immunopathol 41: 305
56. Zinkernagel RM (1988) Virus triggered AIDS is a T cell mediated immunopathology: prevention by tolerance or by treatment with anti-CD8 antibodies. Immunol Today 9: 370