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NOTES

Impaired Resistance to Bacterial Infection After Tumor Implant Is Traced to Lactic Dehydrogenase Virus

PETER F. BONVENTRE,* H. CURT BUBEL, J. GABRIEL MICHAEL, AND ALLEN D. NICKOL

Department of Microbiology, University of Cincinnati Medical Center, Cincinnati, Ohio 45267

A BALB/c mouse-passaged methylcholanthrene-induced fibrosarcoma tumor caused severe impairment of resistance to systemic listeriosis. Depressed resistance expressed immediately after tumor implantation was traced to inadvertent association of tumor with lactic dehydrogenase virus. Tumor cured of virus was totally inactive.

The lactic dehydrogenase-elevating virus (LDV) is a ubiquitous biological agent which establishes chronic, asymptomatic infections in mice (10, 13, 15). The inconspicuous presence of the lactic dehydrogenase agent is relevant to a broad range of biological investigations because it may be unknowingly transmitted with mouse-passaged materials such as body fluids, transplantable tumors, and oncogenic virus preparations (1, 5, 9, 12, 19). Thus, the varied and significant alterations which LDV may induce in the host pose an interpretive hazard in murine experimental systems under scrutiny. A letter to the editor recently published in *Science* (14) cautioned investigators that the virus may alter many immunological and physiological parameters and urged that the presence of LDV in mouse-passaged materials be monitored.

During the initial stages of an investigation designed to evaluate the impact of a growing solid tumor on the native resistance of athymic (nu/nu) and heterozygote (nu/+) mice to bacterial and viral infections, we became aware of the potential effects that LDV may have on the interpretation of our findings. Preliminary experiments suggested that subcutaneous implantation of a methylcholanthrene-induced fibrosarcoma (Meth A) into mice caused a rapid and significant loss of resistance to challenge with the facultative intracellular bacterium *Listeria monocytogenes*. In view of several previous reports which indicated that implanted tumors may modify host defense mechanisms (4, 8, 16–18), we considered our observations to be confirmatory rather than novel. Analysis of the phenomenon, however, revealed that the tumor per se was without measurable effect and that depressed resistance to systemic infection was the direct result of LDV which had inadvertently become associated with the tumor during mouse passage.

The Meth A tumor was maintained in ascites form in BALB/c mice by weekly serial transfers. Solid tumor growth was initiated by injection of 10^7 Meth A cells subcutaneously in the dorsum of the animal. Mice challenged intravenously with 10^4 colony-forming units of *L. monocytogenes* 1 day after tumor implantation were severely impaired in native resistance, as estimated by numbers of viable units in the liver 48 h after challenge (Fig. 1). This assay provides an accurate index of resistance to listeria infection (7, 8), and we have confirmed that the number of colony-forming units in the livers of mice 48 h after challenge is directly correlated with 50% lethal dose values determined at 7 days after challenge. Thus, BALB/c, heterozygote, and athymic mice were severely compromised 1 day after the tumor inoculation, and differences in liver colony-forming units in tumor-bearing mice suggested a 100- to 1,000-fold increase in susceptibility to listeria infection. These data are comparable to those reported previously in which the rapid loss of host resistance after injection of tumor cells into syngeneic mice was attributed to a low-molecular-weight substance of tumor cell origin (8). On the basis of the observations to be described, there is a strong possibility that the effect was in fact caused by inadvertent presence of LDV in the tumors used in those studies. On the other hand, as noted previously, immunosuppressive factors derived from tumor cells have been described by several investigators, and thus suppressive substances of tumor cell origin cannot be discounted. It would be wise in our judgment, however, for investigators to repeat such experiments with tumors proven to be free of LDV.

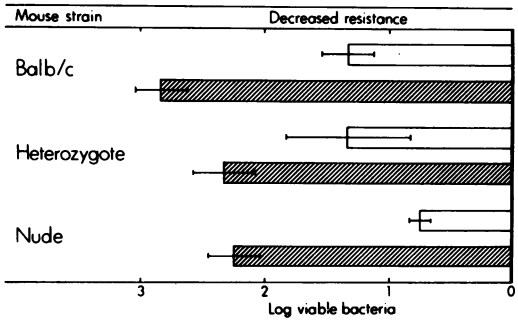

FIG. 1. *Decrease in resistance of immunocompetent and athymic mice to L. monocytogenes 1 day after Meth A tumor implantation. BALB/c-derived Meth A tumor, obtained originally from Alan Kaplan, Medical College of Virginia, Richmond, was maintained by ascites passage in BALB/c mice. Solid tumors were initiated by subcutaneous injection of 10^7 viable, washed ascites-derived tumor cells in a volume of 0.1 ml of phosphate-buffered saline. Tumor-bearing and non-tumor-bearing control animals were challenged intravenously with 2.0×10^4 colony-forming units of L. monocytogenes 24 h after tumor implantation. Spleens and livers of 48-h-infected animals were excised aseptically, homogenized, and assayed for viable organisms. Resistance is expressed as the difference in $\log_{10}$ viable bacteria per gram of organ between infected non-tumor-bearing animals and infected tumor-bearing animals ± standard error of the mean. The origin (0) represents the normalized $\log_{10}$ viable bacteria in infected, non-tumor-bearing animals. The histograms, spleens (open bars) and livers (crosshatched bars), indicate greater numbers of bacteria in the organs of tumor-bearing mice as compared to non-tumor bearers.*

When Meth A-tumor-bearing and non-tumor-bearing mice in our animal colony were screened for plasma lactic dehydrogenase levels, a 5- to 10-fold increase in enzyme levels occurred only in mice bearing the tumors. Groups of normal BALB/c mice previously screened as being free of LDV were injected with either the Meth A tumor or with undiluted stock LDV (obtained from the American Type Culture Collection). Serum from such mice was transferred to LDV-free recipients, and these were subsequently monitored for serum lactic dehydrogenase activity. Since both the LDV-infected and the tumor-bearing mice yielded sera which caused elevated lactic dehydrogenase enzyme levels in recipient mice, we concluded that the Meth A tumor had become contaminated with the lactic dehydrogenase-elevating viral agent during serial passage.

The buoyant density of the virus (1.168 g/cm^3) associated with the Meth A tumor is consistent with that of LDV (2), but attempts to visualize the agent by electron microscopy were unsuccessful. Further studies were conducted to differentiate the effects of LDV on host resistance from those induced by the tumor per se. The virus-contaminated Meth A tumor was cultured in vitro for 6 weeks to eliminate the virus. The capacity of the cured tumor to impair host resistance to listeria infection was ablated (Fig. 2). Inoculation of LDV into mice which were subsequently challenged after 24 h with *L. monocytogenes* resulted in a depression of host resistance comparable to that obtained with the virus-contaminated Meth A tumor. Tumor cell extracts and serum from mice receiving the virus-contaminated tumor also depressed resistance to listeria infection. Virus-free materials were without measurable effect on host resistance. As a definitive experiment to show that the lactic dehydrogenase-elevating agent was responsible for the lowered resistance of mice, we reconstituted the cured Meth A tumor with the LDV viral isolate and determined the effect of the reassociated complex on resistance to *L. monocytogenes* (Fig. 3). The data show unequivocally that reassociation of tumor with the transmissible lactic dehydrogenase-elevating agent reestablished the capacity to impair resistance to listeria. Depression of host resistance was evident for 2 days subsequent to implantation of virus-associated tumor or inoculation of LDV in absence of tumor. Thereafter normal resistance to listeria infection was restored.

Several conclusions may be drawn from these data: (i) the Meth A tumor used in our early experiments had become inadvertently associated with a transmissible agent with characteristics of LDV; (ii) implantation of the virus-contaminated Meth A tumor caused a rapid, severe depression of resistance to *L. monocytogenes* in both immunocompetent (BALB/c and heterozygote) and athymic mice; (iii) the capacity to elevate serum lactic dehydrogenase and to depress host resistance was lost after in vitro culture of the Meth A tumor; (iv) inoculation of an LDV of known origin (American Type Culture Collection) depressed host resistance to *L. monocytogenes* in a manner indistinguishable from the original virus-contaminated tumor; (v) the capacity to elevate serum lactic dehydrogenase and depress host resistance to infection was restored when the cured tumor was reassociated with the transmissible viral agent; (vi) LDV is responsible for the marked impairment of host defense and susceptibility to systemic bacterial infection. The Meth A tumor per se is without measurable effect.

The basis for the LDV suppression of resistance observed is most likely an impairment of macrophage-mediated antibacterial immunity.

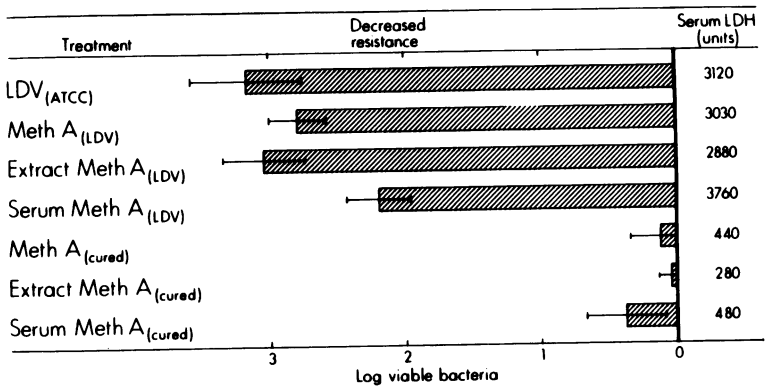

FIG. 2. *Effect of LDV, tumors, and tumor-associated preparations on host resistance to L. monocytogenes and lactic dehydrogenase serum levels in BALB/c mice. Resistance to infection was measured, as described in Fig. 1, 24 h after treatment as follows. LDV (ATCC) was obtained from American Type Culture Collection: 0.1 ml of undiluted stock was inoculated subcutaneously. Meth A (LDV) (our designation for the original virus-contaminated tumor): solid tumors were initiated by subcutaneous implantation of 10^7 viable, washed ascites-derived tumor cells. Extract Meth A (LDV) was prepared by sonication of a Meth A (LDV) cell suspension to achieve total cell disruption; an extract equivalent to 10^7 cells was inculated subcutaneously. Serum Meth A (LDV) was obtained from mice utilized as ascites tumor cell donors; the serum was diluted 1:1,000, and 0.1 ml was inoculated subcutaneously. Meth A (cured) was obtained from Meth A (LDV) cultured 6 weeks in vitro to eliminate virus; the resulting Meth A (cured), readapted to ascites growth, was used for the initiation of solid tumors and the production of extract Meth A (cured) and serum Meth A (cured). Liver and spleen yielded similar results, but only data for the liver are presented. One day after inoculation of one of the above preparations, mice were bled from the retro-orbital venus plexus, and 0.1 ml of serum diluted 1:1,000 was injected intraperitoneally into normal LDV-free, BALB/c mice. Serum lactic dehydrogenase was assayed in these mice 3 days post-serum transfer according to a quantitative ultraviolet procedure (kit no. 340-UV; Sigma Chemical Co., St. Louis, Mo.) based on the spectrophotometric method of Wroblewski and La Due (20). The concentrations of lactic dehydrogenase are expressed as international units.*

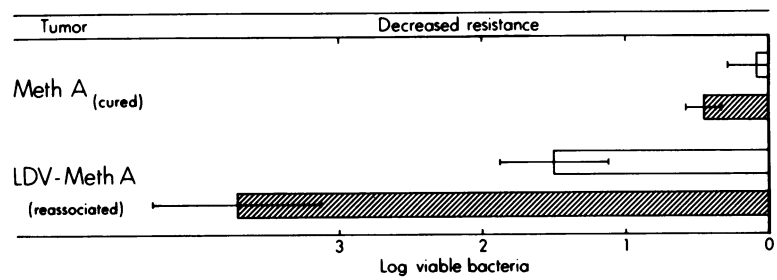

FIG. 3. *The capacity to induce decreased resistance to L. monocytogenes is recovered after reassociation of the Meth A (cured) tumor with the transmissible agent derived from the Meth A (LDV) tumor. Resistance was measured 1 day after tumor implantation into BALB/c mice as described in Fig. 1. The agent derived from the serum of a Meth A (LDV) tumor-bearing mouse was transmitted by serial transfer of serum. One-tenth milliliter of serum diluted 1:1,000 was transferred daily for 5 days by subcutaneous injection in BALB/c mice which were free of LDV. Reassociation of the viral agent and tumor was accomplished by a final subcutaneous transfer of 0.1 ml of 1:1,000-diluted serum to mice simultaneously injected with 5×10^7 Meth A (cured) tumor cells intraperitoneally. The resulting ascites tumor cells were used 7 days later for initiation of the reassociated LDV-Meth A tumor complex.*

L. monocytogenes is a facultative intracellular pathogen, the growth of which is initially suppressed by liver macrophages (7). This early bacterial containment of Kupffer cells is of pivotal importance in the outcome of this infection (6, 7). It is also established that LDV replicates within macrophages in vitro (3) and in vivo (11). Thus, it is likely that early events in LDV infection alter tissue macrophage functions and lead to the observed impaired resistance. The fact that the effect is transient in spite of continuing high virus titers in the blood (9, 10, 13, 15) represents an unresolved paradox. Indeed, our recent observations show that establishment of

chronic LDV infection of BALB/c mice confers enhanced resistance to listeria infection. Failure of athymic mice with chronic LDV infection to develop heightened antibacterial resistance suggests T-lymphocyte involvement (manuscript in preparation).

This brief report establishes for the first time that a lactic dehydrogenase-elevating contaminant of a mouse-passaged tumor is responsible for suppression of native host resistance to bacterial infection. It is emphasized, however, that the fundamental importance of the study is within the wider context of the admonition conveyed by Riley et al. (14). Host alterations induced by LDV, such as the one reported here, may be ascribed erroneously to implanted tumors and thus negate the validity of experiments otherwise meticulously conducted. The ease with which LDV becomes associated with tumors serially passaged in mice must be appreciated. It would appear unconscionable if investigators failed to consider this agent as a potential confounding factor.

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