RAPID COMMUNICATION

Necrosis of Adipose Tissue Induced by Sequential Infections With Unrelated Viruses

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Vaccinia virus infection in mice previously infected with and immune to lymphocytic choriomeningitis virus resulted in a clinical illness which neither virus alone induced. The main pathologic finding was extensive fat necrosis with a cellular infiltrate suggestive of delayed type hypersensitivity. Vaccinia virus titers in adipose tissue of clinically ill mice were not higher than those in relevant control groups. This indicates that an unusual virus-induced disease can arise in an animal with a history of unrelated virus infection, and that this disease may be due to an altered host response to infection. The experimental model presented here suggests that chronic inflammation and necrosis of a given tissue may depend on sequential infection with two viruses, neither of which would be capable of inducing such a lesion. (Am J Pathol 1985, 120:173–177)

VERY LITTLE attention has been given to the fact that a host after recovery from one virus infection may not respond to infection with a second virus in the same way as a host receiving the second virus infection only. It has been shown that a viral infection may reactivate an earlier, latent infection,[1–3] but the phenomenon that we are presenting here is entirely different. Sequential infection with two unrelated viruses (lymphocytic choriomeningitis virus [LCMV] and vaccinia virus [VV] results in a new, wholly unexpected lesion, such as neither virus alone would induce, namely, fat necrosis.

Immunopathology characterized by a T-cell–dependent leptomeningitis is evident in mice infected intracranially with LCMV.[4,5] In this infection, the lesions resemble delayed-type hypersensitivity (DTH) reactions, comprising mostly macrophages, some lymphocytes, and a relatively low number of granulocytes. In VV-infected mice virus-specific cytotoxic T lymphocytes (CTLs) are induced, but VV-infected mice usually do not have severe immunopathologic lesions. We report here that an unusual disease involving DTH-like lesions in adipose tissue occurs in mice during sequential infections with the unrelated viruses, LCMV and VV.

Materials and Methods

Mice

Six to 7-week-old C3H/St male mice were immunized to LCMV by intraperitoneal injection with 8×10^4 plaque-forming units (PFU) of LCMV, strain Armstrong. They were boosted 1 month later with 10^6 PFU

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Table 1—Virus-Induced Disease After Sequential Infection in Mice

Animals*	Number of mice: Diseased† / Tested	Number of mice: Dead	Necrotic lesions in adipose tissue‡	Titers (log_{10} PFU/g)‖ of VV in pelvic fat tissue: Day 3 after VV infection	Titers (log_{10} PFU/g)‖ of VV in pelvic fat tissue: Day 7 after VV infection
Normal control	0/6	0	no (0/2)§	NA¶	NA
MEM-hyperimmune	0/6	0	no (0/3)	NA	NA
LCMV-hyperimmune	0/115	0	no (0/5)	NA	NA
LCMV-hyperimmune + MEM	0/6	0	no (ND)	NA	NA
MEM-hyperimmune + VV	0/3	0	no (ND)	NA	NA
LCMV-acute	0/10	0	no (0/4)	NA	NA
VV-acute	0/40	0	no (0/9)	5.3 ± 0.2 (3)	5.1 ± 0.5 (7)
LCMV-hyperimmune + VV	41/58	5	yes (6/10)	5.4 ± 0.2 (6)	4.5 ± 0.5 (12)

* The immunization of mice was done as described in Materials and Methods.
† Clinical illness was defined by lethargy, ruffled fur, and squinting or closed eyes.
‡ Gross examination of adipose tissue.
§ Adipose tissues were fixed in 10% buffered formalin, cut, embedded in paraffin, sectioned, stained with H&E, and examined microscopically. The numbers in parentheses are the number of animals with necrotic lesions over the number of animals examined.
‖ VV was titrated on vero cell monolayers using a 2-day plaque assay.
¶ NA, not applicable; ND, not determined.

of LCMV, and then held for another 4–6 weeks, at which time there was no detectable LCMV in the organs or detectable LCMV-specific CTL. These mice were designated as LCMV-hyperimmune. The LCMV-hyperimmune mice were next challenged intraperitoneally with 10^7 PFU of VV, Strain WR (LCMV-hyperimmune +VV). For the acute LCMV or VV infection of mice, one injection of 8×10^4 PFU LCMV or 10^7 PFU VV was given intraperitoneally. Each injection was given in a 0.1-ml volume. The mice were sacrificed on days 3, 5, and 7 after infection.

Histology

All tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 5 μ, and stained with hematoxylin and eosin (H&E).

Plaque Assays

Adipose tissue, spleens, and livers from the different groups of animals were homogenized and titrated for virus. VV was titrated on vero cell monolayers with the use of a 2-day plaque assay, and LCMV was titrated in a 4-day plaque assay on baby hamster kidney (BHK) cell agarose suspensions, on which VV fails to form plaques.[6]

Results and Discussion

More than two-thirds of the 58 LCMV-hyperimmune mice that received an acute VV challenge became clinically ill, as indicated by lethargy, ruffled fur, and squinting or closed eyes. Approximately 9% of these mice died 5–6 days after VV infection; and those surviving beyond the seventh day recovered, with no further signs of clinical illness. No illness or death was ever observed in LCMV-hyperimmune mice challenged with minimal essential medium (MEM) only, in mice hyperimmunized with MEM and then challenged with VV, or in mice undergoing normal acute LCMV or acute VV infections (Table 1). Further, no illness was observed in animals hyperimmunized with VV and challenged with LCMV.

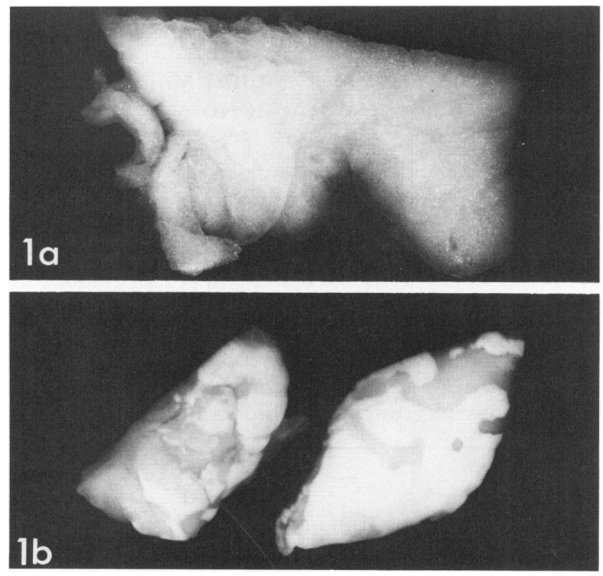
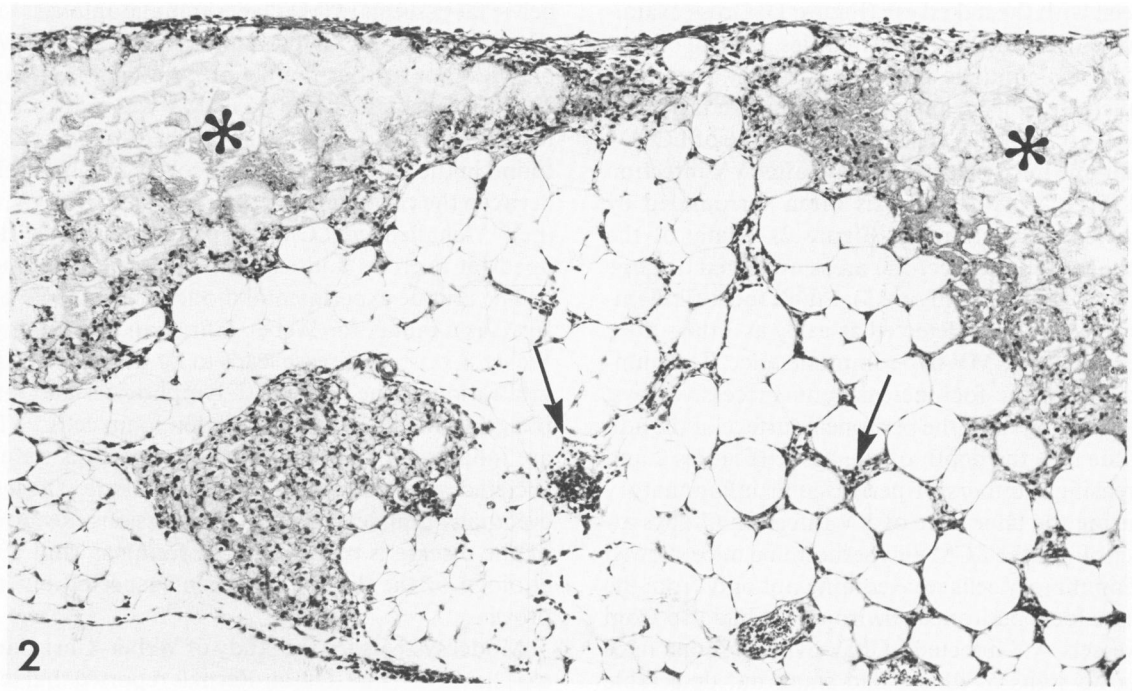
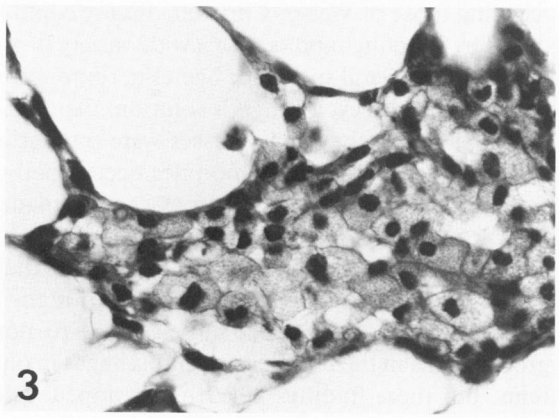
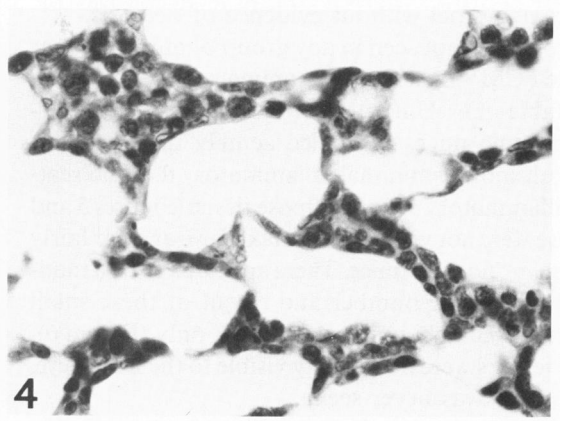


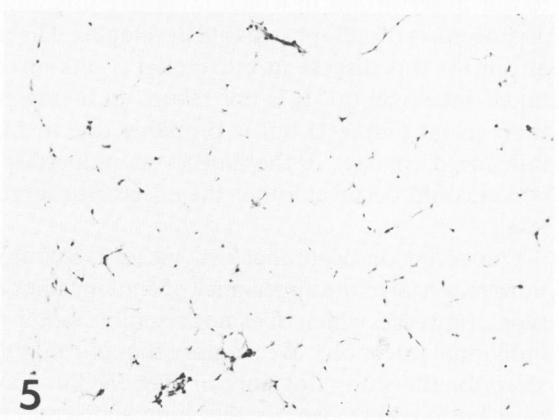
Figure 1—Gross appearance of formalin-fixed pelvic fat body. **a**—LCMV-hyperimmune control. (×2.5). **b**—Vaccinia virus-infected LCMV-hyperimmune mouse. Note the chalky-white areas of necrosis. (×2.5)

At autopsy, the clinically ill mice had chalky-white areas, some of which extended up to 1.5 cm in diameter,









Figures 2–6—Pelvic adipose tissue after virus infection (H&E). **Figure 2**—Day 7 after acute VV infection of LCMV-hyperimmune (Day 7 L-H); necrotic areas are indicated by the *asterisks*, and the *arrows* show inflammatory cells. (×100) **Figure 3**—Lipid-laden macrophages (foam cells) from Day 7 L-H. (×550) **Figure 4**—Mononuclear leukocyte infiltration of Day 5 L-H. (×550) **Figure 5**—LCMV-hyperimmune; it is indistinguishable from normal controls. (×85) **Figure 6**—Day 7 after acute VV infection; the *arrow* points to inflammatory cells. (×100)

in the pelvic, mesenteric, and perirenal fat. These were easily seen with the naked eye (Figure 1). Gross examination revealed no other abnormalities. Several organs (pelvic fat, brain, muscle, heart, lung, kidney, liver, pancreas, intestine, spleen, and aorta [for brown fat]) were examined histologically. The pelvic fat tissue of LCMV-hyperimmune mice sacrificed 7 days after VV infection showed large areas of necrosis often surrounded by mononuclear-cell infiltrates (Figure 2). Many of the macrophages around necrotic areas appeared as large lipid-laden foam cells (Figure 3). Small foci of inflammatory cells could be detected as early as 3 days after VV-infection of LCMV-hyperimmune mice. The number and size of the foci increased on successive days, mainly extending from the peritoneal surface of the adipose tissue into the depth of the tissue (Figures 2 and 4). Increasing numbers of perivascular inflammatory cuffs during the later state of VV infection (7 days after infection) of the LCMV-hyperimmune mice showed that inflammatory cells were coming not only from the immediate local peritoneal environment but also from blood vessels. VV-infected LCMV-hyperimmune mice showing no signs of illness had small but detectable inflammatory foci without evidence of necrosis.

Necrosis was not seen in any group of animals other than the LCMV-hyperimmune animals challenged with VV (Table 1). Untreated normal mice, LCMV-hyperimmune mice, and mice acutely infected with LCMV all showed minimal inflammatory foci and scattered inflammatory cells in adipose tissue (Figures 5 and 6). These were not visible to the naked eye and are fairly common in "normal" mice. There appeared to be a modest increase in the number and extent of these small foci in mice acutely infected with VV only (Figure 6), but the lesions were not usually visible to the naked eye, and necrosis was never seen.

Fat necrosis suggests the possibility of pancreatic disease.[7] Histologic examination of the pancreas (as well as of the aforementioned major organs) showed no abnormalities.

Adipose tissue, spleens, and livers from the different groups of animals were titrated for virus. At 3 days after infection, VV titers were slightly higher in the spleen and liver in mice receiving VV only (4.1 $\pm$ 0.16 and 3.3 $\pm$ 0.13 $\log_{10}$ PFU, respectively) than in LCMV-hyperimmune mice receiving VV (3.5 $\pm$ 0.29 and 2.2 $\pm$ 0.28 $\log_{10}$ PFU, respectively). The VV titers of pelvic adipose tissue in both sets of animals were very high, more than ten times greater than in other organs (Table 1). At 7 days after infection, VV could no longer be recovered from spleen and liver (<1.8 $\log_{10}$ PFU/g tissue), whereas it remained at high titers in the fat (Table 1). No LCMV could be found in spleens, livers, or pelvic fat tissue in LCMV-hyperimmune animals. In VV-challenged LCMV-hyperimmune mice, LCMV-like plaques (not further characterized) were found in only 2 of 15 spleens tested, and no LCMV was recovered from fat tissue. Thus, whereas no marked differences were found in the amount of virus recovered from fat tissue between the two groups of mice infected with VV, only the VV-challenged LCMV-hyperimmune mice developed fat necrosis and extensive DTH-like lesions.

The unique experimental model presented here may serve as a model for Weber–Christian disease in man. Weber–Christian disease leads to fat tissue necrosis and infiltration of the tissue with lymphocytes and macrophages, which become lipid-laden foam cells.[8,9] There are inflammatory nodules in subcutaneous tissue associated with infiltration of adipose tissue. The lesions eventually heal, leaving pitlike depressions. Recurrences of the disease is reported to be frequent, and a virus etiology of the disease has been suspected but never proven.

Model systems for the study of Weber–Christian disease have been rare. Duran-Reynals reported that lesions recalling those of Weber–Christian disease could be induced by injecting rabbits with a wide variety of agents, such as VV, several species of bacteria, tumor cells, tissue extracts, and even Ringer's solution.[9] Autopsies revealed that grossly visible masses were present in the abdominal fat and fibrotic nodules occasionally were embedded in the muscle. The masses were made of a capsulelike structure with a central cavity full of necrotic adipose tissue. He also reported that hemorrhage of varying degrees was present in masses in many locations, including fat tissue and muscles. We have not seen gross lesions in the muscles or hemorrhages in our systems, but these findings nevertheless appear relevant to our observations in which DTH-like inflammation and necrosis of adiopose tissue developed. The mechanism for this disease in our model is unknown and under investigation. It is not related to increased VV titers in fat (Table 1) but is probably due to LCMV-influenced changes in the anti-VV immune response, which could occur at either the effector or target cell level.

Our results indicate that two successive viral infections may lead to the appearance of a totally unexpected type of disease, which does not resemble either of the individual infections. We propose that our model may serve for the study not only of Weber–Christian disease, but possibly also of other human diseases of unknown etiology, which may have resulted from a sequence of infections with more than one virus.

References

1. Lemon SM, Hutt LM, Huang YT, Blum J: Simultaneous infection with multiple herpesviruses. Am J Med 1979, 66:270–276
2. Whitaker-Dowling P, Youngner JS: Vaccinia rescue of VSV from interferon-induced resistance: Reversal of translation block and inhibition of protein kinase activity. Virology 1983, 131:128–136
3. Oldstone MBA, Aoki T, Dixon FJ: Activation of spontaneous murine leukemia virus-related antigen by lymphocytic choriomeningitis virus. Science 1971, 174:843–845
4. Cole GA, Nathanson N, Prendergast RA: Requirement for θ-bearing cells in lymphocytic choriomeningitis virus-induced central nervous system disease. Nature 1972, 283:335–337
5. Gilden DH, Cole GA, Nathanson N: Immunopathogenesis of acute central nervous system disease produced by lymphocytic choriomeningitis virus: II. Adoptive immunization of virus carriers. J Exp Med 1972, 135:874–889
6. Pulkkinen AJ, Pfau CJ: Plaque size heterogeneity: A genetic trait of lymphocytic choriomeningitis virus. Appl Microbiol 1979, 20:123–128
7. Tedeschi CG: Pathological anatomy of adipose tissue. Handbook Physiol 1965, 5:141–168
8. Christian HA: Relapsing febrile nodular nonsuppurative panniculitis. Arch Int Med 1928, 42:338–351
9. Duran-Reynals F: A necrotizing disease in rabbits affecting fatty and muscular tissues. Yale J Biol Med 1945, 18:583–594