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Age-Related Loss of Lyt-1,2 Cells in C58 Mice Results in Susceptibility to Lactic Dehydrogenase Virus-Induced Polioencephalomyelitis

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C58 mice (aged ≥5 months) are susceptible to age-dependent polioencephalomyelitis, a paralytic central nervous system disease induced by lactic dehydrogenase virus. Susceptibility results from the loss of protective T cells. Data are presented showing a positive correlation between the age-related loss of Lyt-1,2 cells and the development of susceptibility to neuroparalytic lactic dehydrogenase virus infection.

Neurovirulent strains of lactic dehydrogenase virus (LDV) elicit a paralytic central nervous system disease in strain C58 mice (1, 8, 10; D. M. Bentley and R. E. Morris, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, T84, p. 249). The disease is characterized clinically by flaccid hind limb paralysis and histologically by nondemyelinating neuronal destruction with mononuclear infiltration and microglial proliferation in the areas of the lesions (7). The natural aging process or immunosuppressive treatment with cyclophosphamide or X-ray irradiation render C58 mice susceptible to disease induction (4, 9). Duffey et al. (3) demonstrated that susceptibility to age-dependent polioencephalomyelitis (ADPE) results from a loss of protective T cell function; the protective T cell population resides in the spleen. Protective activity begins to wane as animals reach 5 months of age and is almost completely absent in 1-year-old mice (9). We have recently shown that an age-related physical deletion of T cells is responsible for the development of susceptibility and have reported that three Lyt alloantigen-defined T cell subsets (Lyt-1, Lyt-1,2, and Lyt-2 cells) are required to protect cyclophosphamide-treated mice against disease induction (2).

With the knowledge that three antigenically and functionally distinct T cell subpopulations are required to protect animals against the disease, we designed these studies to determine which subset(s) was lost during the natural aging process. The procedures used to select the Lyt-defined subsets from nylon-wool-enriched splenic T cell populations have been described in detail previously (2), and, as before, all animals used in these studies were purchased from the Jackson Laboratories, Bar Harbor, Maine.

Mice (aged 11 months) of which >80% are naturally susceptible to ADPE (Table 1) were reconstituted with splenic T cell populations from disease-insusceptible, syngeneic donors by intravenous injection of cells on days 14 and 7 before challenge with 10^5 50% infectious doses of LDV. Transfer of Lyt-1 and Lyt-2 cells failed to protect aged indicator mice against disease induction (Table 1). Transfer of either Lyt-1^+ (Lyt-1 and Lyt-1,2) or Lyt-2^+ (Lyt-2 and Lyt-1,2) cells, however, protected 100 and 87.5% of the indicator mice, respectively (Table 1). Taken together, these results suggested that the Lyt-1^+,2^+ (Lyt-1,2) population was required to reconstitute the protective response of aged, naturally susceptible mice. To test this hypothesis, Lyt-1,2 cells were enriched with a plate adherence procedure in which cells were first selected for Lyt-2 expression and then, after 4 h of incubation at 37°C (in RPMI 1640 medium containing 10% fetal bovine serum and 10 mM HEPES [*N*,2-hydroxyethylpiperazine-*N*′,2-ethanesulfonic acid] buffer) to allow for shedding of cell surface bound antibody, for the expression of Lyt-1 alloantigen. This procedure yielded a population in which cells were >93% positive for the expression of both Lyt alloantigens as judged by direct immunofluorescence. No paralysis was observed in animals that were reconstituted with the doubly selected cells (Table 1), confirming that the age-related decline in protective response was attributable to a loss of Lyt-1,2 cells.

Although it is certain that a loss of Lyt-1,2 cells results in the development of susceptibility

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TABLE 1. Age-related loss of the Lyt-1,2 subset

Group	Cells transferred[a]	No. of animals paralyzed/ total[b]	% Protection
1	Lyt-1^{+}[c]	0/8	100.0
2	Lyt-2^{+}[c]	1/8	87.5
3	Lyt-1 + Lyt-2[d]	0/7	100.0
4	Lyt1[b] + Lyt2[c]	7/8	12.5
5	None	9/11	18.2

[a] Positive selection was used for groups 1 through 3; negative selection was used for group 4 (2).

[b] Results are the compilation of at least two separate experiments. Indicator mice were infected with 10^6 50% infectious doses of line I_b leukemia-derived LDV (10), and animals were scored as positive on the basis of development of flaccid hind limb paralysis.

[c] A total of 10^7 cells from 4- to 6-week-old donors were transferred in each of 2 intravenous injections on days 14 and 7 before LDV infection.

[d] A total of 2×10^6 cells from 4- to 6-week-old donors were transferred in each of 2 intravenous injections on days 14 and 7 before LDV infection. Cells were >93% positive for expression of both Lyt alloantigens. Final cell recovery was <5% of the initial cell number.

to neuroparalytic LDV infection, a concomitant loss of Lyt-1 or Lyt-2 cells (or both) may also occur. The inability of Lyt-1 and Lyt-2 cells to protect aged mice against disease induction, however, demonstrates that the Lyt-1,2 population is required to reconstitute the protective response of aged mice.

It has become evident that many T-dependent immune responses require interaction between antigenically and functionally distinct T cell subsets. This is particularly true for antigen-specific suppressor T cell pathways (for review, see references 5 and 6). Although functionally characterized, the ADPE protective network most probably possesses suppressor activity and serves to prevent the induction of a virally induced, neuropathogenic inflammatory response in the central nervous system. In the ADPE protective network, as in many suppressor T cell pathways, the efferent suppressor T cells express the Lyt-1^{-},2^{+} phenotype. Whether Lyt-2 cells are derived from the Lyt-1,2 pool by activational or differentiative processes is as yet undetermined. The use of a complex in vivo model for these studies does not permit direct examination of the fate of Lyt-1,2 cells. If, however, Lyt-2 efferent protective cells merely differentiated from the Lyt-1,2 population without need for antigenic stimulus and interaction with the other T cell subsets, it would have been expected that the transfer of Lyt-2 cells alone would have been protective. These results are, therefore, interpreted as additional evidence for a three-cell network in which antigenically and functionally distinct T cell subsets interact to bring about the activation of a protective Lyt-1^{-},2+ population which, as far as can be determined by Lyt phenotyping, has already differentiated.

It remains to be determined whether the loss of Lyt-1,2 cells, some of which may be undifferentiated precursors, is a general phenomenon which might account for age-related abberrations in immune function. The mechanism by which these cells are lost is also, at present, unknown. Recent studies suggest that C58 mice mount a substantial thymocytotoxic autoimmune response which might, at least in part, lead to the deletion of cells in the protective network (D. M. Bentley and W. H. Murphy, manuscript in preparation). In summary, these studies demonstrate that a loss of Lyt-1,2 cells, one of three essential T cell subsets involved in protection against neuropathogenic LDV infection, leads to the development of susceptibility to ADPE and emphasize how the loss of one T cell subpopulation can drastically alter the outcome of immune responses which involve multiple cellular interactions.

LITERATURE CITED

1. **Bentley, D. M., V. A. Guckian, J. D. Stinnett, and R. E. Morris.** 1982. Failure to demonstrate a role for line I_b tumor-associated surface antigen in the etiology of age dependent polioencephalomyelitis. Mol. Immunol. **19**:983–989.
2. **Bentley, D. M., and R. E. Morris.** 1982. T cell subsets required for protection against age dependent polioencephalomyelitis of C58 mice. J. Immunol. **128**:530–534.
3. **Duffey, P. S., O. A. Lukasewycz, D. Martinez, and W. H. Murphy.** 1976. Pathogenic mechanisms in immune polioencephalomyelitis: quantitative evaluation of protective and pathogenic effects of lymphoid cells. J. Immunol. **116**:1332–1336.
4. **Duffey, P. S., D. Martinez, G. D. Abrams, and W. H. Murphy.** 1976. Pathogenic mechanisms in immune polioencephalomyelitis: induction of disease in immunosuppressed mice. J. Immunol. **116**:475–481.
5. **Germain, R. N., and B. Benacerraf.** 1980. Helper and suppressor T cell factors. Springer Semin. Immunopathol. **3**:93–127.
6. **Germain, R. N., and B. Benacerraf.** 1981. Hypothesis: a single major pathway of T-lymphocyte interactions in antigen-specific immune suppression. Scand. J. Immunol. **13**:1–10.
7. **Lawton, J. W. M., and W. H. Murphy.** 1973. Histopathology of immune polioencephalomyelitis in C58 mice. Arch. Neurol. **28**:376–370.
8. **Martinez, D. N., M. A. Brinton, T. G. Tachovsky, and A. H. Phelps.** 1980. Identification of lactic dehydrogenase virus as the etiologic agent of genetically restricted age dependent polioencephalomyelitis of mice. Infect. Immun. **27**:979–987.
9. **Murphy, W. H., M. R. Tam, R. L. Lanzi, M. R. Abell, and C. Kauffman.** 1970. Age dependence of immunologically induced central nervous system disease in C58 mice. Cancer Res. **30**:1612–1617.
10. **Pease, L. R., and W. H. Murphy.** 1980. Co-infection by lactic dehydrogenase virus and C-type retrovirus elicits neurologic disease. Nature (London) **286**:398–400.