

CROSS-REACTIVE KILLER T LYMPHOCYTES IN A FLAVIVIRUS INFECTION

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Summary. — Splenocytes from mice immunized intravenously with one dose of Langat or Yellow fever viruses were cytotoxic for target cells infected with virulent tick-borne encephalitis (TBE) virus; cytotoxicity was measured by ^{51}Cr release from target L929 cells. No significant lysis of the TBE virus-infected target cells with splenocytes from Sindbis virus-infected mice was noted.

Key words: *Flavivirus; cross-reactivity; T lymphocytes; cytotoxicity*

The mechanisms of the long-lasting cross-resistance after primary infection with live attenuated flaviviruses have been incompletely delineated. The immunizing effect is judged upon protection from lethal disease after challenge within certain flavivirus subgroups, e. g. the TBE complex of viruses (Mayer, 1963, 1975), or even cross-protection with some other members of the *Flavivirus* genus. Because the role of low, often undetectable levels of virus-neutralizing antibodies, which are rather type-specific, is hardly decisive and the flavivirus group-reactive haemagglutinin (HA) antibodies are not contributory (Price *et al.*, 1963), the importance of cell-mediated immunity (CMI) in the resistance of the above type was suggested (Mayer and Mitrová, 1977). In infections with viruses, in particular those released by budding, the cell membrane-associated neoantigens are effective in the induction of CMI reactions. Actually the recognition of TBE viral antigens by lymphokine-producing lymphocytes (Ly) from TBE convalescents (Mayer *et al.*, 1976) and from mice experimentally infected with the attenuated Langat E5 "14" virus (the TBE complex) was definitely shown (Gajdošová and Mayer, 1978). Another Ly subpopulation, the virus-immune thymus-dependent killer cells (TKC), generated in the same model, was revealed recently (Gajdošová *et al.*, to be published) by ^{51}Cr -release cytotoxicity assay. In TKC recognition of virus-associated antigens, operating only in syngeneic systems, the "self" genes of the host (here the H-2K locus) are clearly involved (Doherty *et al.*, 1978). TKC were identified as T-Ly by rabbit antimouse thymocyte serum. We observed cross-reactivity in TKC, an event possibly relevant for some of the above phenomena.

Table 1. Cytotoxicity of spleen effector cells from mice immunized with Langat, Yellow fever, or Sindbis virus on TBE virus-infected L cells

Effector cells from mice immunized with	% ⁵¹ Cr release (mean ± S.D.) from target cells uninfected	% ⁵¹ Cr release (mean ± S.D.) from target cells infected
Langat E5"14"	26.10 ± 1.8	86.10 ± 2.7
Yellow fever 17D	22.61 ± 1.6	89.12 ± 0.5
Sindbis (<i>Alphavirus</i>)	28.15 ± 1.6	33.77 ± 2.2
Uninfected mice	28.37 ± 0.9	27.54 ± 1.6
Spontaneous ⁵¹ Cr release from L929 cells	26.28 ± 0.6	24.35 ± 1.6

Splenocytes from mice immunized with flaviviruses (Langat, Yellow fever) showed significant cytotoxicity against target cell type ($P < 0.001$).

Subadult C3H inbred mice were given 10^3 plaque forming units (PFU) of Langat E5"14", Yellow fever (YF) 17D or Sindbis virus intravenously. Splenic cells, isolated on the 6th day after inoculation, were added into L929-cell tube cultures uninfected or infected with the "204" strain of TBE virus, western subtype (obtained from Dr. M. Grešíková, Institute of Virology), labelled with 0.18 MBq of ⁵¹Cr ($\text{Na}_2 \text{}^{51}\text{CrO}_4$ with a specific activity of 3.7–14.8 MBq per mg chromium), at an effector: target cell ratio of 100 : 1. After overnight incubation at 37 °C, the activity in supernatants, cells and their freezing-thawing lysates was measured in a gamma radiation counter Tesla 54.

From experiments, performed in triplicate, it is apparent (Table 1) that cytotoxic reactivity of TKC, generated in mice given the YF 17D virus, diverges from the serologically defined antigenic determinants of this strain, relatively distant from TBE virus within the *Flavivirus* genus and seems to approach the HA cross-reactivity between them.

No significant lysis of the TBE virus-infected target cells with Ly from Sindbis virus-infected mice was noted. It may be hypothesized that infection with viruses of one group, e. g. flaviviruses, sharing a similar general structure and certain antigenic and biological traits, may result in expression of a common novel antigen on the plasma membrane of such cells. But the structural substrate of the virus-induced modification of the cell surface, recognized at binding sites by TKC, needs to be produced by interaction of the virus and host genomes. Additional studies will be required to find out whether TKC comprise at least two Ly populations, e. g. one specific for virus causing primary infection and the other cross-reactive within the *Flavivirus* genus as well.

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