THERMAL INACTIVATION OF THE PARTIALLY PURIFIED MURINE TRANSFER FACTOR

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Transfer factor (TF) is specified (*1*) as a component of dialysable leukocytes lysate (DLL) with capacity to transfer specific cutaneous delayed hypersensitivity (CDH) reactions from a sensitized donor to a non-sensitized recipient.

In rather qualitative assays (skin tests; leukocytes migration inhibition, LMI) there was shown (*2, 3*) that higher temperatures abolished TF activity, but the effect of intermediate temperatures remained inconclusive. Human TF, specific for PPD (*M. tuberculosis*), was fractionated on Sephadex G-25 and inactivated after 30 min at 80 °C, in part at 56 °C, but not at 37 °C. CDH transfer was reported as not influenced by incubation of TF for 2 hr at 37 °C, slightly after 30 min at 56 °C, but completely destroyed after 30 min at 100 °C. Moreover, in DLL a material distinct from the TF activity carrying component was found (*4*), causing non-specific intradermal reaction.

We studied the TF activity inducing cytotoxic T cells, specific against the Flavivirus group antigen in the ^{51}Cr release test using tick-borne encephalitis (TBE) virus infected target cells. DLL was prepared from spleen cells of mice, immunized with Langat virus (*5*). The activity was concentrated by 100-times by ethanol precipitation (*6*) and subsequent Sephadex G-25 chromatography. Absorbance profile showed three peaks, with the activity detectable in the second peak.

Aliquots of this material containing 4×10^6 TF units, were dissolved in one ml of bidistilled water and incubated in an ultrathermostat water bath ($\pm$ 0.01 °C). At temperature of 30 °C, the starting TF activity was lowered after 6 hr by one, after 11.5 hr by two and after 19 hr by 4 $\log_{10}$ units. The decrease of the TF activity titre was linear, showing a single-hit character. The velocity constant of inactivation ($S/S = e^{-kt}$) at 30 °C was 0.485, i.e. 0.1904 $\log_{10}$ units/hr. No activity was detected in samples after 60 min at 56 °C or 80 °C. If the same specific TF activity was quantitatively measured in the crude DLL, subjected to 37 °C, the decrement of the starting titre (10^4 TF units) represented after 24 hr 3 $\log_{10}$ units only (*5*). Accordingly, presence of carrier substances protective for the TF activity cannot be excluded in DLL, as suggested also by the slope and more rapid inactivation rate of partially concentrated and purified materials at 30 °C.

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