SPECIFIC INDUCER FACTOR PURIFIED FROM SPLENOCYTIC DIALYZATES OF GOAT IMMUNIZED WITH JAPANESE ENCEPHALITIS VIRUS

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Two opposite antigen-specific substances were found in the transfer factor (TF, *1*).Most of the clinical trials of TF therapy have employed impure TF. TF contains in variable amounts substances exerting specific suppressoric effects on the interactions of TF with cells of the immune system. Suppressoric activity does not only block TF's inducing activity, but abrogates also the response of already immune cells to the specific antigen. In order to improve the efficiency of TF preparations, we have attempted to isolate a specific inducer factor (SIF).

Goat that was used as a source of spleen to prepare specific transfer factor (STF) was immunized with live Japanese encephalitis virus (JEV, Gao strain). STF was prepared by the conventional procedure. JEV 51-8 anti-idiotype antibody (JEV 51-8 Ab_2, *2*) was purified by DEAE-dextran 25. The reverse passive haemagglutination assay showed that JEV 51-8 Ab_2 had JEV antigen activity. High purity of JEV 51-8 Ab_2 was identified by electrophoresis. In a liquid phase system (0.01 mmol/l, phosphate-buffered saline pH 7.4, PBS) JEV 51-8 Ab_2 as a ligand was mixed with STF and incubated overnight at 4 °C. The mixture was dialyzed repeatedly against sterile PBS at 4 °C until no absorbing material (280 nm) was released. The nondialyzable complex SIF-JEV 518 Ab_2 was redialyzed against sterile bidistilled water at 20 °C and the dialyzable material represented SIF. The purity of SIF was assayed by high performance liquid chromatography through McH-5, which yielded a single peak in UV region. SIF content comprised only 18.98 % of STF.

In order to develop a reproducible system to assess passive transfer of TF *in vitro*, was introduced a rapid colorimetric assay based on the leukocyte adherence inhibition (LAI, *3*). A tetrazolium dye 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) has been used for assaying the number and activity of non-adherent leukocytes. SIF showed strong inhibitory response to JEV antigen as compared with STF ($P<0.01$). Mice which received SIF showed significant footpad responses to JEV antigen. The histology of footpads showed marked infiltrations with mononuclear cells that were consistent with delayed-type hypersensitivity. The TF preparations were injected into mice in an attempt to protect them against a lethal challenge with JEV. It was shown that SIF significantly protected ($P<0.05$) recipients against JEV challenge, whereas STF failed to do so (*4*).

Our findings indicate that for obtained highly pure, specific and potent SIF for induction or augmentation of specific cell-mediated immunity, the present approach may be useful. The availability of purified SIF should facitate its analysis, the understanding of its role in immune function and its potential clinical application.

References

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