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T-CELLS OF VIRUS-INFECTED MICE PRODUCE A LYMPHOKINE WHICH ACTIVATES THE AUTOREACTIVITY OF INTACT MOUSE LYMPHOCYTES

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Summary. – The role of lymphokines was estimated in induction of autoreactive T-cells during Langat virus infection in mice. It was shown that *in vitro* cultured splenocytes from virus-infected animal containing autoreactive lymphocytes (ARL) spontaneously produce a lymphokine which is capable to activate the autoreactivity of lymphocytes derived from the spleen of intact syngeneic mice. The capacity of this lymphokine to activate the autoreactivity of acceptor cells within 2 hr was demonstrated by local graft-versus host reaction (GVHR) in the donor-recipient system. According to their surface markers (theta-antigen expression, absence of immunoglobulins) the lymphokine activating autoreactivity (LAA) producers may belong to T-lymphocyte population. Autoreactivity could be induced by the lymphokine only if the LAA producers and acceptors were compatible by the major histocompatibility complex antigens.

Key words: autoreactive T-lymphocytes; Langat virus; lymphokine activating autoreactivity

Induction of autoreactive T-lymphocyte (ARL) is characteristic for the pathogenesis of infectious process induced by some viruses (Semenov *et al.*, 1982). As shown for flaviviruses, the negative regulation of this type of autoimmune response involves the participation of a serum-blocking factor and of suppressor T-cells activated in the course of infection (Khozinsky and Semenov, 1980; Khozinsky and Semenov, 1983). The mechanisms of ARL expansion in the virus-infected host are obscure. It is assumed that ARL accumulate by means of proliferation only. The precursor clone is differentiated into effector cells of the autoimmune pathologic process.

Under conditions of syngeneic adaptive transfer to intact recipients of donor splenocytes containing virus-induced ARL, they accumulate in the body of the recipients which develop a clinically manifested autoimmune process (Vargin *et al.*, 1987). We used this model to elucidate the role of cell-mediated and

humoral mechanisms of ARL expansion. In this report we demonstrate the capacity of T-cell population containing virus-induced ARL to produce LAA activating autoreactivity of intact mouse spleen T-cells *in vitro*.

Mice were given intraperitoneal injections of 10^4 LD_{50}/0.03 ml of Langat virus (TP-21 strain) passaged in suckling mouse brain. Six days later when ARL were detected in the spleen, these were removed from the infected donors, splenocytes were isolated, cultured (1–5 x 10^7 cells/ml) in RPMI-1640 medium supplemented with glutamine (2 mmol/l), 7 % foetal calf serum, HEPES (10 mmol/l) and 2-mercaptoethanol (5x 10^{-5} mmol/l). After 2 hr cultivation at 37 ^{0}C the culture fluid was freed from cells and detritus by centrifugation at 2000 rev/min for 20 min, and the supernatant was irradiated with an ultraviolet B-15.1 lamp from a distance of 15 cm for 90 min to inactivate the possibly present virus and subsequently used for treatment of intact mouse splenocytes. Intact mouse spleen cells (1x10^6 cells/ml) were grown for 2 or 24 hr at 37 ^{0}C in the supernatant obtained as described. Splenocytes treated with culture fluid were washed 3 times in medium 199 supplemented with 2 % foetal calf serum.

Autoreactive lymphocytes were tested by local GVHR in a syngeneic mouse system (Zschiesche and Vargin, 1976); the material was injected in a dose of 15 x 10^6 cells/0.1 ml to the animal's footpad. Six days later the popliteal lymph nodes of hind paws were isolated and weighed. The indices of correlation between the mass of lymph node of the paw where the material tested was injected, to the mass of contralateral popliteal node were calculated. For evaluating local GVHR 10 mice for each group were used. Statistical analysis of the results, identification of cells producing lymphokine which activates autoreactivity and effector cells induced by it were performed using immune cytolysis with anti-theta-sera (ATS) and antisera to mouse immunoglobulins (ASMG) as previously described (Vargin and Semenov, 1980).

We have shown (Varing *et al.*, 1987) that in the course of intravenous transfer to intact syngeneic recipients of donor splenocytes containing Langat virus-induced ARL the spleen of recipients exhibited ARL which activity was significantly enhanced. Subsequent karyologic analysis of splenocytes derived from BALB/c female recipients (H-2^d) which were given 6 days prior examination the ARL-enriched splenocytes from syngeneic males (H-2^d) did not confirm the notion that ARL proliferation was the major mechanism of lymphocyte autoreactivity. No ARL with the karyotype of male donors was found among 100 metaphase T-lymphocytes in the spleen of female recipients.

As follows from Table 1 the culture fluid of mouse splenocytes from Langat virus-infected mice was capable to induce the autoreactivity of splenocytes derived from intact syngeneic mice. To induce autoreactivity determined in local GVHR in the syngeneic system, acceptor cells should be treated at least for 2 hr. Splenocytes treated with culture fluid for 24 hr exhibited a more marked activity. The supernatant of splenocyte cultures of uninfected mice including those cultured in the presence of inactivated Langat virus did not

Table 1. Characterization of lymphokine activating autoreactivity (LAA) and its producers

Group	SP donors		Producers	The material examined	GVHR*
	uninfected	Langat virus infected			
1	CBA	-	SP	C + SP $(CBA)_2$	1.06 (09-1.16)
2	CBA**	CBA	SP	C + SP $(CBA)_2$	0.98 (0.92-1.17)
3	CBA	CBA	SP	C + SP $(CBA)_2$	1.8 (1.5-2.05)
4	-	CBA	SP	C + SP $(CBA)_{24}$	2.25 (2.1-2.4)
5	-	CBA	ASP	C + SP $(CBA)_2$	1.08 (0.95-1.13)
6	-	CBA	NASP	C + SP $(CBA)_2$	1.9 (1.6-2.2)
7	-	CBA	NASP + ATS	C + SP $(CBA)_2$	1.1 (0.95-1.2)
8	-	CBA	NASP + ASMG + GPC	C + SP $(CBA)_2$	1.75(1.5-2.05)
9	-	CBA	SP	C + SP $(CBAxC57B1/6)_{F1}$	1.7 (1.36-1.92)
10	-	CBA	SP	C + SP (C57B1/6)	0.98 (0.86-1.26)

Footnote. SP — splenocytes, ASP — adherent splenocytes, GPC — guinea pig complement, C — cytokine (culture fluid of splenocytes),
SP $(CBA)_2$ and SP $(CBA)_{24}$ — splenocytes were cultivated at 37 ^{0}C with C for 2 and 24 hr, respectively. For further abbreviations see the text.
*Ratio of popliteal lymph node mass of the hind paw from mouse receiving the material compared to the mass of contralateral popliteal node (the confidence intervals are given in brackets, p — 0.05);
**SP were cultured in the presence of UV-inactivated Langat virus.

reveal such an activity (compare groups 1 and 2, 3, 4). Glassnonadherent splenocytes (GNAS) of infected animals were capable of LAA production. Glass-adherent splenocytes of Langat virus-infected mice did not reveal LAA-type factor production in culture (compare groups 3,5, and 6). LAA-producing GNAS were shown to be sensitive to ATS treatment but resistant to ASMG (compare groups 3, 7, and 8). This finding indicates the expression of theta-antigen on cells tested and the absence of immunoglobulins. The latter implies that LAA producers can be considered T-lymphocytes.

To enable T-lymphocytes to produce LAA, protein synthesis is apparently required since treatment of LAA producers with actinomycin D (1 μg/ml) prevents the release of LAA (data not shown). It should be emphasized that in contrast to the majority of lymphokines described (Smith, 1986) the factor detected in our experiments exhibited its activity either in the syngeneic system or if LAA producers and acceptors shared the antigens of the major histocompatibility complex. The lymphokine produced by splenocytes of Langat virus-infected CBA mice (H-2^k) activate the autoreactivity of splenocytes derived from CBA or hybrid (CBAx C57B1/6)F_1 (H-2^{kb}) mice, but not from C57B1/6 mice (H-2^b) as determined in syngeneic GVRH using CBA (CBAxC57B1/6)F_1 and C57B1/6 mice, respectively (compare groups 3, 9, 10). However, when splenocytes from mouse lines C57B1/6 or (CBAxC57B1/6)F_1 were used as LAA producer as well as acceptor cells, the splenocytes were shown to acquire autoreactive properties (data not shown).

The capacity of T-lymphocytes from infected animals to produce LAA is regarded for an important mechanism of induction of T-cell autoreactivity in the virus-infected host; this mechanism may function together with the proliferative reaction of ARL.

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