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THE TISSUE DISTRIBUTION OF SPONTANEOUS CELL-MEDIATED CYTOTOXICITY EFFECTOR LYMPHOCYTES IN SWINE

By

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In previous papers, we have described spontaneous cell-mediated cytotoxicity (SCMC) against target cells infected with transmissible gastroenteritis virus (TGEV) by peripheral blood lymphocytes (PBL) and small intestinal intra-epithelial lymphocytes (IEL) from young adult pigs (Cepica and Derbyshire, 1983; 1984a). Spontaneous cell-mediated cytotoxicity against cells infected with Aujeszky's disease virus has also been described (Martin and Wardley, 1984). These authors found that lung lavage cells, as well as PBL, were capable of causing cytolysis. Spontaneous cell-mediated cytotoxicity against a human myeloid leukemia (K–562) cell line has been studied more extensively. This reaction was demonstrated with porcine PBL by Koren, Amos and Kim (1978) and subsequently confirmed by Charley, Petit, Laude and La Bonnardière (1983), Norley and Wardley (1983) and by Martin and Wardley (1984). Kim, Huh, Koren and Amos (1980) found that effector cells against K–562 targets were lacking in porcine spleen, thymus, mesenteric lymph nodes, bone marrow and tonsil, but these lymphoid tissues have not been examined for SCMC effector activity against virus-infected target cells. In the present study, we examined suspensions of porcine lymphocytes from thymus, spleen, mesenteric lymph nodes and Peyer's patches, as well as PBL and IEL, for SCMC effector activity against TGEV-infected target cells.

Effector cells were obtained from five outbred Yorkshire pigs, approximately 6 months old, which lacked TGEV neutralizing antibodies. Suspensions of PBL and IEL were prepared as previously described (Cepica and Derbyshire, 1983; 1984a). Spleen, thymus, mesenteric lymph nodes and Peyer's patches were gently minced and passed through a fine mesh screen and the cells were collected in Hanks' balanced salt solution (HBSS). After they were washed twice in HBSS, the cells were resuspended to appropriate concentrations in complete RPMI–1640 medium (Cepica and Derbyshire, 1984a). The viability of the isolated cells was shown to be greater than 90 per cent by trypan blue exclusion. Spontaneous cell-mediated cytotoxicity chromium (^{51}Cr) release assays were performed as described by Cepica and Derbyshire (1983) with PK–15 cells persistently infected with TGEV as targets, at an effector:target cell ratio of 50 to 1. Appropriate controls were included as before, and all determinations were made in triplicate except for the controls for spontaneous and total ^{51}Cr release, which were done in sextuplicate. The amounts of ^{51}Cr

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released were determined as counts per minute (cpm) in an automatic gamma counting system. The results of the SCMC assays were expressed as percentage specific ^{51}Cr release, computed as 100 × [(mean cpm effector + target cells) − (mean cpm target cells only) ÷ (mean total releasable cpm) − (mean cpm target cells only)].

The results of the SCMC ^{51}Cr release assays on the various lymphocyte populations are shown in Table 1. Peripheral blood lymphocytes and IEL were the only cells that mediated significant specific ^{51}Cr release. While consistently of SCMC were mediated by IEL than by PBL, the differences between mean values of percentage specific ^{51}Cr release (PBL mean = 44·4 ± 14·5 S.D.; IEL mean = 50·0 ± 13·8 S.D.) were not significant at the 5 per cent level by the *t*-test. The lymphoid cells derived from the thymus, spleen, mesenteric lymph nodes and Peyer's patches were devoid of cytolytic activity, except for the production of marginal amounts of cytolysis by splenic lymphocytes from pig numbers 3 and 4 (Table 1). This finding is in agreement with the report by Kim *et al.* (1980) in which K–562 cells were used as targets, although in their study neither IEL nor Peyer's patch lymphocytes were tested. However, in a study of SCMC of gut mucosal cells in the guinea pig (Arnaud-Battandier, Bundy, O'Neill, Bienenstock and Nelson, 1978) SCMC effector activity was demonstrated in IEL but not in Peyer's patch lymphocytes. The high SCMC activity in porcine IEL may be important in TGE and other enteric viral infections in which the intestinal epithelial cells adjacent to the IEL are the primary targets for the virus and we recently obtained some experimental evidence that SCMC effector cells among the IEL may contribute to resistance to TGE (Cepica and Derbyshire, 1984b).

TABLE 1

TISSUE DISTRIBUTION OF SCMC EFFECTOR LYMPHOCYTES AGAINST PK-15 CELLS INFECTED WITH TGEV

	Percent specific SCMC ^{51}Cr release					
	Source of effector lymphocytes					
Pig number	*Peripheral blood*	*Intestinal epithelium*	*Thymus*	*Spleen*	*Mesenteric lymph node*	*Peyer's patch*
1	65	70	−0·2	2·0	0·8	0·8
2	51	57	0·7	1·5	−0·7	1·3
3	41	44	0·2	3·2	0·3	2·0
4	26	34	−0·6	6·5	1·2	0·6
5	39	45	0·3	0·1	−0·2	−0·8

SUMMARY

Peripheral blood lymphocytes, intraepithelial lymphocytes from the small intestine and lymphocytes from the thymus, spleen, mesenteric lymph nodes and Peyer's patches from 5 young adult pigs were used as effector cells in a spontaneous cell-mediated cytotoxicity chromium release assay against PK–15 cells persistently infected with transmissible gastroenteritis virus as targets. Both peripheral blood and intraepithelial lymphocytes caused marked specific

chromium release, while the lymphocytes from the remaining tissues were inactive in spontaneous cell-mediated cytotoxicity.

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