Functional T cells in rabbits gut mucosal lymphocytes¹

D. P. SINGAL, M. O'NEILL, R. CLANCY, AND J. BIENENSTOCK

From the Host Resistance Program, Departments of Pathology and Medicine, McMaster University, Hamilton, Ontario, Canada

SUMMARY Functional studies of Peyer's patch and gut mucosa lymphocytes were performed in mixed lymphocyte culture reaction (MLC), an *in vitro* measure of the capacity of T cells to recognize and respond to cell surface alloantigens. The data demonstrate the functional effectiveness of both these mucosal lymphocyte populations in one-way MLC, both as responding as well as stimulating cells. Both the responding and stimulating capacities of these cells in MLC were almost the same as observed for lymphocytes obtained from either spleen or thymus. We conclude that functional T cells exist in both rabbit gut lamina propria and Peyer's patches with approximately the same quantitative capacity to respond in the MLC.

Mucous membranes which provide the first line of defence against potentially harmful microorganisms share important structural and functional characteristics. The lamina propria of these membranes is very rich in competent immunoglobulin producing cells, most of which contain immunoglobulin A (IgA). Most mucous membranes also possess well-developed lymphoid aggregates. Peyer's patches are unique islands of lymphoid tissue in the mammalian small intestine. Both lamina propria and Peyer's patches contain T and B cells (Raff, 1969; Kincade *et al.*, 1970; Pernis *et al.*, 1970; Raff and Owen, 1971; Rudzik *et al.*, 1975). Although there are T lymphocytes in the lamina propria of rabbit gut mucosa, their number is small and no evidence exists as to the functional capacity of these cells in this or any other species. Although B cells from Peyer's patches can proliferate and mature into antibody-forming cells (Craig and Cebra, 1971), there are conflicting reports concerning the immunocompetence of Peyer's patch T cells. Heim *et al.* (1972) and Perey and Guttman (1972) have shown that Peyer's patches from mice and rats were not capable of producing a graft *vs* host (GVH) reaction in recipient allogenic animals. Using an *in vitro* system to evaluate GVH, Kerckhaert *et al.* (1973) found that rat Peyer's patches lack fully immunocompetent T cells. On the other hand, Levin *et al.* (1974) reported that guinea-pig Peyer's patch cells respond to allogenic cells *in vitro* in mixed lymphocyte culture

reaction (MLC) and further reported that guinea-pig and rat Peyer's patch cells could produce a GVH reaction in appropriate recipients.

Because of the usefulness of the rabbit in models of mucosal immunity and since we have described the distribution of thymus-specific antigen in various lymphoid tissues in this animal (Rudzik *et al.*, 1975), we attempted to define the functional capabilities of T cells from Peyer's patch and gut mucosa. The functional studies of Peyer's patch and gut mucosa lymphocytes were performed in mixed lymphocyte culture reaction (MLC), an *in vitro* measure of the capacity of T cells to recognize and respond to cell surface alloantigens. By performing such experiments in parallel with spleen and thymus lymphocytes from the same donor animals the relative functional capabilities of mucosal lymphocytes could be legitimately compared.

Methods

Tissues were obtained from New Zealand white rabbits aged 14 to 18 weeks. The technique for obtaining lymphocytes from the spleen, thymus, Peyer's patch, and the gut mucosa have already been described (Rudzik *et al.*, 1975). The cells obtained consisted of more than 95% lymphocytes and had a viability of greater than 90% by the dye-exclusion test.

The one-way MLC test was performed as described by us before using 100×10^3 responding lymphocytes and 100×10^3 mitomycin treated stimulating lymphocytes in 0.1 ml volume (Singal *et al.*, 1975). Heat inactivated (at 56°C for 30 min)

¹Supported by Research Grants from the Medical Research Council of Canada.

Received for publication 3 February 1976

heparinized pooled plasma from several rabbits was added in 0.05 ml volume per culture. Since 5-fluorocytosine (5 FC) prevents fungal contamination, 5 µg/ml 5 FC were added to the final volume (Levin *et al.*, 1974). All tests were done in triplicate. All the results that were obtained are given as stimulation ratios (SR), cpm in the combination AB_m divided by cpm in the combination AA_m.

Results

To determine the capacity of T cells from these lymphoid tissues to act as responder cells, one-way MLC was performed with these cells from a rabbit as responder cells and mitomycin treated spleen cells from another rabbit as stimulating cells (Table 1). In experiment I, SR obtained for thymus cells as responder cells was 20.49, for spleen cells 10.59, Peyer's patch cells 4.54, and for gut mucosal cells 4.81. However, in the other two experiments, no differences were found in the SR values obtained with these lymphocytes as responder cells.

One-way MLC was performed with spleen cells from a rabbit as responding cells and mitomycin treated spleen, thymus, Peyer's patch, and gut mucosal lymphocytes as stimulating cells (Table 2). It is evident that mitomycin treated lymphocytes from Peyer's patch and gut mucosa produced as much proliferative response in allogenic spleen cells

Cells*	CPM†	SR‡
Experiment I		
S ₁ × S _{1m}	428 ± 1.5	1.00
S ₁ × S _{2m}	4533 ± 372.5	10.59
T ₁ × T _{1m}	170 ± 21.1	1.00
T ₁ × S _{2m}	3484 ± 273.9	20.49
P ₁ × P _{1m}	1140 ± 30.5	1.00
P ₁ × S _{2m}	5180 ± 469.7	4.54
G ₁ × G _{1m}	384 ± 80.0	1.00
G ₁ × S _{2m}	1847 ± 350.3	4.81
Experiment II		
S ₁ × S _{1m}	109 ± 26.0	1.00
S ₁ × S _{2m}	2768 ± 211.8	25.39
T ₁ × T _{1m}	126 ± 16.0	1.00
T ₁ × S _{2m}	2796 ± 202.0	22.19
P ₁ × P _{1m}	146 ± 18.5	1.00
P ₁ × S _{2m}	4131 ± 874.5	28.29
Experiment III		
T ₁ × T _{1m}	204 ± 18.8	1.00
T ₁ × S _{2m}	808 ± 8.5	3.96
P ₁ × P _{1m}	542 ± 159.3	1.00
P ₁ × S _{2m}	1835 ± 801.5	3.39
G ₁ × G _{1m}	240 ± 56.5	1.00
G ₁ × S _{2m}	880 ± 16.5	3.67

Table 1 One-way MLC in rabbit with spleen, thymus, Peyer's patch and gut mucosa lymphocytes as responding cells

*S, spleen cells; T, thymus cells; P, Peyer's patch cells; G, gut cells; m, mitomycin treated.

†Gross counts per minute per tube ± SEM.

‡SR, ratio of experimental cpm to control cpm.

Cells	CPM	SR
Experiment I		
S ₁ × S _{1m}	385 ± 16.5	1.00
S ₁ × S _{2m}	4574 ± 912.0	11.88
S ₁ × T _{2m}	4382 ± 128.0	11.38
S ₁ × P _{2m}	5872 ± 339.5	15.25
S ₁ × G _{2m}	5285 ± 643.4	13.73
Experiment II		
S ₁ × S _{1m}	372 ± 89.5	1.00
S ₁ × T _{2m}	2083 ± 0.5	5.59
S ₁ × P _{2m}	2736 ± 775.5	7.35
S ₁ × G _{2m}	3003 ± 679.5	8.03

Table 2 One-way MLC in rabbit with spleen, thymus, Peyer's patch, and gut mucosa lymphocytes as stimulating cells*

*See Table 1.

as did mitomycin treated spleen or thymus lymphocytes. These experiments show quite clearly the capability of these cells to stimulate in the MLC.

Table 3 gives the data of experiments in which Peyer's patch or gut mucosa lymphocytes were used as responders and stimulators in one-way MLC. It is evident that SR obtained in Peyer's patch × Peyer's patch_m combination was not different from that obtained for Peyer's patch × spleen_m combination. Similar results were obtained for gut mucosa lymphocytes.

Cells	CPM	SR
Experiment I		
P ₁ × P _{1m}	355 ± 25.4	1.00
P ₁ × S _{2m}	1487 ± 170.3	3.43
P ₁ × P _{2m}	1432 ± 162.9	3.31
Experiment II		
G ₁ × G _{1m}	538 ± 34.6	1.00
G ₁ × S _{2m}	2146 ± 679.8	3.99
G ₁ × G _{2m}	1387 ± 0.5	2.58

Table 3 One-way MLC in rabbit with Peyer's patch and gut mucosa lymphocytes*

*See Table 1.

Discussion

The data in the present investigation demonstrate the functional effectiveness of Peyer's patch and gut mucosa lymphocytes in one-way MLC both as responding as well as stimulating cells. Furthermore, the responding and the stimulating capacities of these cells in MLC were almost the same as observed for lymphocytes obtained from either spleen or thymus.

Our results in MLC support the observation made in guinea-pig Peyer's patch cells by Levin *et al.* (1974). However, our results at first sight seem to

contrast with those reported by Kagnoff and Campbell (1974), which indicate inability of mouse Peyer's patches to mount cell-mediated cytotoxicity. This inability to evoke cytotoxicity was reversed by the addition of macrophages or 2-mercaptoethanol, which suggests that some adherent cell types may be absent from their mouse Peyer's patch cell preparation. Since both the MLC and the cell mediated cytotoxicity assay measure the ability of T cells to respond to cell-surface alloantigens, it appears that rabbit Peyer's patches may harbour a functioning cell type absent in mice. However, the 'killer' effector cells, necessary for a cytotoxic assay and in some way different from the proliferating cells, are not measured by the MLC (Bach *et al.*, 1973). Therefore, it is possible that this cytotoxic effector function is deficient in mouse Peyer's patches and not the ability of Peyer's patch cells to recognize and respond to cell-surface alloantigens. Indeed, mouse Peyer's patch cells have been shown to possess T helper-cell function (Katz and Perey, 1974).

The initial responding cell in the MLC seems to be mainly the T cell. By using electrophoretically fractionated spleen T and B cells, Andersson *et al.* (1973) found that T cells alone respond to allogenic cells, while B cells alone did not respond, a result which was later confirmed by Boehmer (1974). Also, Andersson *et al.* (1973) found that at the most 5% of mitotic cells in the allogenic MLC test are of B cell origin. One might, therefore, expect a lower MLC with spleen, Peyer's patch and gut mucosa responding cells as compared with MLC with thymus responding cells, since 20% of spleen cells, 17% of Peyer's patch cells, and 11% of gut mucosa cells possess a thymus-specific antigen (Rudzik *et al.*, 1975). However, in the present investigation, no differences were observed in the responding capacity of cells obtained from thymus, spleen, Peyer's patch, and gut mucosa in MLC. It seems probable that the T cells need to be present for induction of the MLC *in vitro*, and B cells may also participate in the MLC interaction. We conclude that functional T cells exist in rabbit gut lamina propria and Peyer's patches.

References

- Andersson, L. C., Nordling, S., and Häyry, P. (1973). Proliferation of B and T cells in mixed lymphocyte cultures. *Journal of Experimental Medicine*, **138**, 324-329.
- Bach, F. H., Segall, M., Zier, K. S., Sondel, P. M., Alter, B., and Bach, M. L. (1973). Cell mediated immunity: separation of cells involved in recognitive and destructive phases. *Science*, **180**, 403-406.
- Boehmer, H. (1974). Contribution of lymphocyte subpopulations to allogeneic and syngeneic mixed lymphocyte reaction. Proceedings of the 8th Leucocyte Culture Conference, Uppsala, Sweden. Academic Press: New York, London. pp.289-295.
- Craig, S. W., and Cebra, J. J. (1971). Peyer's patches: an enriched source of precursors for IgA-producing immunocytes in the rabbit. *Journal of Experimental Medicine*, **134**, 188-200.
- Heim, L. R., McGarry, M. P., Montgomery, J. R., Trentin, J. J., and South, M. A. (1972). Potentials of spleen, lymph node, and Peyer's patches to reconstitute lymphoid tissue and produce graft-versus-host reaction. *Transplantation*, **14**, 418-423.
- Kagnoff, M. F., and Campbell, S. (1974). Functional characteristics of Peyer's patch lymphoid cells. I. Induction of humoral antibody and cell-mediated allograft reactions. *Journal of Experimental Medicine*, **139**, 398-406.
- Katz, D. H., and Perey, D. Y. E. (1973). Lymphocytes in Peyer's patches of the mouse: analysis of the constituent cells in terms of their capacities to mediate functions of mature T and B lymphocytes. *Journal of Immunology*, **111**, 1507-1513.
- Kerckhaert, J. A. M., Benner, R., and Willers, J. M. N. (1973). Cells involved in the graft-versus-host reaction *in vitro*. *Immunology*, **25**, 103-110.
- Kincade, P. W., Lawton, A. R., Bockman, D. E., and Cooper, M. D. (1970). Suppression of immunoglobulin G synthesis as a result of antibody-mediated suppression of immunoglobulin M synthesis in chickens. *Proceedings of the National Academy of Sciences*, **67**, 1918-1925.
- Levin, D. M., Rosenstreich, D. L., Wahl, S. M., and Reynolds, H. Y. (1974). Peyer's patch lymphocytes: demonstration of the integrity of afferent and efferent T-cell function in the guinea-pig and rat. *Journal of Immunology*, **113**, 1935-1941.
- Perey, D. Y. E., and Guttman, R. D. (1972). Peyer's patch cells: absence of graft-versus-host reactivity in mice and rats. *Laboratory Investigation*, **27**, 427-433.
- Pernis, B., Forni, L., and Amante, J. (1970). Immunoglobulin spots on the surface of rabbit lymphocytes. *Journal of Experimental Medicine*, **132**, 1001-1018.
- Raff, M. C. (1969). Theta isoantigen as a marker of thymus-derived lymphocytes in mice. *Nature*, **224**, 378-379.
- Raff, M. C., and Owen, J. J. T. (1971). Thymus-derived lymphocytes: their distribution and role in the development of peripheral lymphoid tissues of the mouse. *European Journal of Immunology*, **1**, 27-30.
- Rudzik, O., Clancy, R. L., Perey, D. Y. E., Bienenstock, J., and Singal, D. P. (1975). The distribution of a rabbit thymic antigen and membrane immunoglobulins in lymphoid tissue with special reference to mucosal lymphocytes. *Journal of Immunology*, **114**, 1-4.
- Singal, D. P., Younglai, E. V., and Naipaul, N. (1975). Inhibition of the mixed lymphocyte culture reaction by plasma from renal transplant recipients. *Tissue Antigens*, **5**, 19-25.