NATURAL KILLER (NK) CELL ACTIVITY AGAINST ENTERIC MURINE CORONAVIRUS MEDIATED BY INTESTINAL LEUKOCYTES

P.S. Carman, P.B. Ernst, K.L. Rosenthal, D.A. Clark,
D.A. Befus and J. Bienenstock

McMaster University
Hamilton, Ontario, Canada

INTRODUCTION

Relatively little is known about local cell mediated immunity (CMI) in the gut and its role in resistance to enteric virus disease. Intraepithelial leukocytes (IEL), because of their location within the epithelium, may be involved in the defense of the gut mucosa against viruses. This study was initiated to assess the endogenous local CMI response of murine mucosal lymphocytes, especially IEL, to an enveloped enteric murine virus. The Yale strain of mouse hepatitis virus (MHV-Y) was chosen for these studies. MHV-Y causes diarrhea in infant mice (1) and has been shown to be highly tropic for the intestinal epithelium (2). It replicates in NCTC-1469 cells which have the $H\text{-}2^k$ phenotype (3).

IEL from conventional mice are heterogenous in phenotype and function. A large population of $Thy\text{-}1^-$, $Lyt\text{-}1^-$, $Lyt\text{-}2^+$ cells is present, but its function is unknown. Most of these $Lyt\text{-}2^+$ cells are granulated. They represent 45 to 55% of the total IEL population (4-7). Less than 15% of IEL have natural killer (NK) activity against YAC-1 tumor cells. The majority of these NK cells have the unusual NK cell phenotype $Thy\text{-}1^+$, $Lyt\text{-}1^-$, $Lyt\text{-}2^-$, $ASGM1^-$, $NK1^-$ (8). Unlike lymphocytes from the lamina propria (LPL), IEL do not bear surface immunoglobulin (5).

J. Mestecky et al. (eds.), *Recent Advances in Mucosal Immunology*
© Plenum Press, New York 1987

METHODS AND RESULTS

Cells from different compartments of the gut-associated lymphoid [IEL, LPL, Peyer's patch (PP) and mesenteric lymph node (MLN)] and spleen (SPL) cells were harvested (8,9) from inbred strains of mice. Because conventional mice were seropositive for MHV-Y, specific pathogen free (SPF) mice were used.

Since all previous IEL studies in our laboratory have used conventional mice, the distribution of surface antigen expression of IEL from conventional and SPF CBA/J mice were initially compared by flow cytometry (Table 1). Approximately 30% of IEL from conventional mice were Thy-1^{+}, Lyt-1^{+}, whereas 80% were Lyt-2^{+}, as has been previously reported (5). Although virtually all SPF-derived cells demonstrated the Ly-5 lymphocyte antigen, with few contaminating Ig^{+} LPL evident, cells from SPF mice expressed less Thy-1^{+}, Lyt-1^{+} and Lyt-2^{+} antigens. This pattern of phenotypic expression by SPF mice is similar to that reported for nude mice (4) and most probably reflects limited antigen exposure for the gut in both groups.

Effector cells from CBA/J mice, syngeneic to the target cell and DBA/2 and A/J mice, allogeneic for the target cell, were assessed for their ability to lyse MHV-Y infected NCTC-1469 cells using standard chromium release assays. The data is expressed as lytic units per 10^6 effector cells (10). The lytic activity reported is virus-specific for it is the difference between virus-infected and uninfected targets. Cell populations harvested from these mice showed strong cytotoxic activity, especially IEL, for the MHV-Y infected target cell (Table 2). The lack of MHC restriction suggests that the cytotoxic cell is an NK cell.

Table 1. Phenotype of IEL from CBA/J mice determined by flow cytometry

	Thy-1.2	Lyt-1.1	Lyt-2.1	ASGM1	Ig[a]	Ly-5
Conventional Mice	38.1±5.4	32.4±4.1	80.0±1.8	11.4±3.0	4.9±0.6	ND
SPF Mice	7.7±0.7	3.4±0.3	46.6±4.1	17.0±3.4	1.1±0.5	92.5±6.2

[a]Surface immunoglobulin.

Table 2. Specific cytotoxicity for MHV-Y infected target cells by different strains of SPF mice

Mouse Strain	IEL	LPL	PP	MLN	SPL
CBA/J (H-2^k)	21.9±1.2	3.3±1.6	4.1±0.8	0.9±0.4	2.3±0.3
DBA/2 (H-2^d)	14.2±0.7	ND	3.1±0.7	0.7±0.2	2.0±0.4
A/J (H-2^a)	4.4±1.4	ND	1.8±0.5	0.3±0.2	0.8±0.1

The lytic activity reported is the difference between virus-infected and uninfected targets calculated for each experiment and expressed as $LU_{20}/10^6$ cells; mean ± SEM.

Intraepithelial leukocytes and spleen cells harvested from SPF CBA/J (H-2^k) mice were assessed using in vitro ^{51}Cr release assays, for their ability to lyse the NK sensitive YAC-1 tumor cell line, syngeneic NCTC-1469 cells acutely infected with MHV-Y and NCTC-1469 cells infected with pichinde virus (PV), an enveloped but non-enterotropic arenavirus.

The MHV-Y NK cell appears not to be the same cell that lysis the classical murine NK target, YAC-1 tumor cells. In cytotoxicity assays (Table 3), effector cells from SPF mice showed low lytic activity to YAC-1 tumor targets. This ineffective YAC-1 tumor cell killing reflects the small numbers of Thy-1^+ cells in IEL preparation from SPF mice. This negligible cytotoxicity is in contrast to the pronounced ability of effector cells, especially IEL, to kill NCTC-1469 cells infected with MHV-Y. The cytotoxic cell appears to be "virus-specific" since it was not lytic for PV infected cells.

Table 3. Cell-mediated lysis of various target cells

Target	IEL	SPL
YAC-1	0.4 ± 0.0	1.0 ± 0.7
MHV-NCTC-1469	21.9 ± 1.2	2.3 ± 0.3
Pichinde-NCTC-1469	0.8 ± 0.8	0.4 ± 0.3

$LU_{20}/10^6$ cells; Mean ± SEM

Table 4. Phenotype of cytotoxic IEL from naive mice determined by complement-mediated lysis

Antisera + Complement	Percentage decrease in lysis
Thy 1.2	17
Lyt 1.1	0
Lyt 2.1	11.5
ASGM1	89

The phenotype of the IEL cytotoxic for MHV-Y infected NCTC-1469 cells was determined using *in vitro* complement-mediated lysis assays. The specific cytotoxicity of the IEL cells was reduced by 89% after treatment with anti-ASGM1 serum and complement (Table 4). Antisera to Thy-1, Lyt-1 and Lyt-2 antigens had little effect on the function of the lytic cells. This data suggests that the MHV-Y killer cell is an $AsGM1^+$ cell.

To confirm this observation, *in vivo* CBA/J mice were treated intravenously with anti-ASGM1 serum at levels previously reported to be effective for the elimination of $AsGM1^+$ cells *in vivo* (11), 24 hours prior to cell harvest. This *in vivo* treatment resulted in a 75% reduction in the *in vitro* lytic activity for MHV-Y infected target cells by IEL (Table 5), as well as an 83% reduction in the lytic activity by spleen cells. These experiments further define the MHV-Y killer cell, found in naive mice, as an $AsGM1^+$ NK cell.

Table 5. MHV-Y specific cytotoxicity following *in vivo* anti-ASGM1 sera

	IEL	SPL
CBA/J ($H-2^k$)		
Untreated	21.9 ± 1.2	2.3 ± 0.3
Treated with anti-ASGM1	5.5 ± 1.5	0.4 ± 0.3
% significant decrease in cytotoxicity	74.8%	82.6%

$LU_{20}/10^6$ cells; Mean ± SEM

CONCLUSION

The *in vitro* and *in vivo* results suggest that a subpopulation of the morphologically and functionally heterogenous IEL compartment is involved in the defense of the gut mucosa to enteric viruses. This population of cells has the phenotype $ASGM1^+$, $Thy\text{-}1^-$, $Lyt\text{-}1^-$, $Lyt\text{-}2^-$, but does not possess the target specificity of classic NK cells (12).

ACKNOWLEDGEMENT

This investigation was supported by the Medical Research Council of Canada.

REFERENCES

1. Barthold, S.W., Smith, A.L., Lord, P.F.S., Bhatt, P.N., Jacoby, R.O. and Main, A.J., Lab Anim. Sci. 32, 376, 1982.
2. Barthold, S.W. and Smith, A.L., Arch. Virol. 81, 103, 1984.
3. Van Loveren, H., Van Der Zeijst, A.M., De Weger, R.A., Van Basten, C., Pijpers, H., Hilgers, J. and Den Otter, W., J. Reticuloendothel. Soc. 29, 443, 1981.
4. Parrott, D.M.V., Mackenzie, S., Mowat, A. McI., Davies, M.D.J. and Micklem, H.S., Ann. N.Y. Acad. Sci. 409, 307, 1983.
5. Petit, A., Ernst, P.B., Befus, A.D., Clark, D.A., Rosenthal, K.L., Ishizaka, T. and Bienenstock, J., Eur. J. Immunol. 15, 211, 1985.
6. Schrader, J.W., Scollay, R. and Battye, F., J. Immunol. 130, 558, 1983.
7. Dillon, S.B. and Macdonald, T.T., Immunol. 52, 501, 1984.
8. Tagliabue, A., Befus, A.D., Clark, D.A. and Bienenstock, J., J. Exp. Med. 155, 1785, 1982.
9. Davies, M.D.J. and Parrott, D.M.V., Gut 22, 481, 1981.
10. Clark, D.A., Phillips, R.A. and Miller, R.G., Cell Immunol. 34, 25, 1977.
11. Habu, S.H., Fukui, H., Shimamura, K., Kasai, M., Nagai, Y., Okumura, K. and Tamaoki, N., J. Immunol. 127, 34, 1981.
12. Carman, P.S., Ernst, P.B., Rosenthal, K.L., Clark, D.A., Befus, A.D. and Bienenstock, J., J. Immunol. 136, 1548, 1986.