Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 9183–9187, December 1986
Medical Sciences

Specific human cytotoxic T cells recognize B-cell lines persistently infected with respiratory syncytial virus

CHARLES R. M. BANGHAM AND A. J. MCMICHAEL

Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Oxford OX3 9DU, United Kingdom

Communicated by James Gowans, August 5, 1986

ABSTRACT **The T-lymphocyte response to respiratory syncytial (RS) virus has been invoked to explain the bronchiolitis and pneumonia caused by RS virus in human infants. However, T cells also appear to play a role in protection against RS virus infection. Although RS virus-specific human lymphocytes have been demonstrated, neither the phenotype nor the function of the lymphocytes was characterized. We describe here the induction of anti-RS virus cytotoxic T lymphocytes, in both bulk culture and restimulated cell lines, from human peripheral blood. Infection of Epstein–Barr virus-transformed human B-cell lines with RS virus *in vitro* readily caused a persistent infection; these cells continued to synthesize RS viral proteins and secrete infectious RS virus 4 months after infection. The persistently infected cells were used both to restimulate cytotoxic-T-cell precursors and as targets for RS virus-specific cytotoxic T cells.**

Respiratory syncytial (RS) virus, a ubiquitous paramyxovirus, is the most important viral cause of lower respiratory tract infection in infants (1) and causes recurrent infections in adults. There is no vaccine widely available against RS virus; a formaldehyde-inactivated RS virus vaccine that underwent clinical trials in the 1960s provided no protection against the infection and tended to exacerbate the disease on subsequent exposure to natural infection (2, 3). Although there is some correlation between antibody titer and protection against RS virus infection in cotton rats (4, 5), mice (6), and humans (7), even the highest titers of anti-RS virus antibody are insufficient to prevent bronchiolitis or pneumonia in some infants (7). Also, Prince *et al.* (8) showed that some cotton rats were almost completely immune to RS virus despite undetectable antibody titers.

These observations suggest that cell-mediated immunity plays a part in limiting RS virus infection. Fishaut *et al.* (9) found that clearance of a persistent RS virus infection in children with severe congenital immunodeficiency was achieved only in those with an intact cell-mediated immune response. RS virus-specific proliferation of human peripheral blood mononuclear cells (PBMCs) has been described (e.g., ref. 10). However, in human studies neither the phenotype nor the function of the cells involved has been investigated. Previous reports have described major histocompatibility complex (MHC) class I antigen-restricted cytotoxic T lymphocytes (CTLs) in the mouse (11), which crossreacted, at the polyclonal level, with all human strains of RS virus tested (12). Here we describe the induction and characterization of RS virus-specific HLA-restricted CTLs in both bulk cultures of T cells and CTL lines derived from human PBMCs. The CTL lines were derived by restimulation of bulk cultures of T cells with Epstein–Barr virus (EBV)-transformed B-cell lines (BCL) persistently infected with RS virus *in vitro*; the same BCLs served as efficient RS virus-specific targets for the CTLs.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Viruses. The A2 strain of RS virus, prepared and assayed as described (11), was used throughout. The virus stock had a titer of 2×10^6 plaque-forming units (pfu) per ml. Influenza A/X31 (H3N2) was grown in chicken egg allantoic cavities, and the allantoic fluid was harvested and stored at −80°C until use; hemagglutination titers ranged between 1:1000 and 1:2000. EBV was maintained in B95-8 cells as described (13).

Anti-Interferon Antisera. Sheep anti-human interferon α (H51) and sheep anti-human interferon γ (H53) were donated by A. Meager (National Institute for Biological Standards and Control, London). Each antiserum had a titer of 5×10^4 interferon-neutralizing units/ml.

Monoclonal Antibodies. mAbs were kindly donated as indicated below. (*i*) Anti-RS virus mAbs: anti-fusion (F) protein (1A12) and anti-G protein (4G4), E. Routledge (Royal Victoria Infirmary, Newcastle, U.K.) (14); anti-nucleoprotein (N) (Mab15) and anti-F protein (Mab11), G. Taylor (Institute for Research on Animal Diseases, Newbury, U.K.) (6); and anti-N (6-3), B. Fernie (Georgetown University Schools of Medicine and Dentistry, Rockville, MD) (15). (*ii*) T-cell surface marker mAbs: anti-CD3 (UCHT1) P. Beverley (University College Hospital, London) (16); anti-CD4 (Leu 3a), R. Evans (Memorial Sloan–Kettering, New York) (17); and anti-CD8 (B941), C. Mawas (Institut National de la Santé et de la Recherche Médicale, Marseilles) (18).

Persistent Infection of BCLs with RS Virus. BCL cells (5×10^6) were infected with RS virus at a multiplicity of infection of 1–2 pfu per cell for 1 hr at 37°C, then resuspended in 4 ml of RPMI/10 [RPMI 1640 (GIBCO) containing 10% fetal bovine serum, penicillin (60 μg/ml), streptomycin (100 μg/ml), and glutamine (300 μg/ml)] and incubated at 37°C in 6% CO_2. These persistently RS virus-infected BCL (RS.BCL) cells were split at a ratio of 1:2 once a week. Infection was confirmed by indirect immunofluorescence staining using anti-F or anti-G mAbs (see above) as first reagent on live cells in suspension or anti-N mAb as first reagent on cells that had been fixed with acetone at −20°C for 5 min. The second reagent was fluorescein isothiocyanate-conjugated goat-anti-mouse immunoglobulin (Sigma).

Assay of Infectious RS Virus Recovered from Culture Supernatants. Two milliliters of culture supernatant was added to 6×10^4 HEp-2 cells in each well of a 24-well plate (Costar, Cambridge, MA). After 36 hr of incubation at 37°C, the monolayers were washed, fixed in 4% paraformaldehyde, and stained by an indirect immunoperoxidase technique using an anti-F mAb (Mab11) as first reagent and a peroxidase-conjugated anti-mouse immunoglobulin (Dako, Santa Barbara, CA) as second reagent.

Abbreviations: BCL, B-cell line; CTL, cytotoxic T lymphocyte; EBV, Epstein–Barr virus; mAb, monoclonal antibody; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cell; pfu, plaque-forming unit(s); RPMI/10, RPMI 1640 medium with 10% fetal bovine serum plus antibiotics and glutamine; RS virus, respiratory syncytial virus.

Immunoprecipitation. RS virus-infected cell lines were labeled with [^{35}S]methionine (100 μCi per 3 × 10^6 cells; 1 Ci = 37 GBq) for 1 hr. Cells were washed in phosphate-buffered saline and lysed in a buffer (19) containing 0.5% (vol/vol) Nonidet P-40 and 1% (wt/vol) Mega 9 (see ref. 49) detergents. The lysate was precleared with 10% (wt/vol) formaldehyde-fixed *Staphylococcus aureus* cells (Cambridge Bioscience, Cambridge, U.K.) and the N protein was precipitated with N-specific mAbs (Mab15 and 6-3), followed by protein A-Sepharose (Pharmacia).

PBMC Preparation. PBMCs were separated from heparinized venous blood from healthy adult volunteers, using lymphocyte-separation medium (Flow Laboratories). No volunteer had received an RS virus vaccine. All volunteers had presumably been infected with RS virus in the past (7).

Tissue Typing. Tissue typing was carried out using standard National Institutes of Health techniques. The HLA types of volunteers used in this study were A2,3 B7,15 DR2,4 (volunteer IH), A25,29 B44 DR1,7 (FG), A1,30 B13,37 DR7 (MG), and A3,24 B15,37 DR6 (PC).

Induction of CTLs. PBMCs (3 × 10^6) were suspended in 300 μl of virus stock for 1 hr at 37°C and then added to 10^7 autologous PBMCs in 8 ml of RPMI/10. After incubation for 6–10 days at 37°C (6% CO_2), these cells (effectors) were either used in a 5-hr ^{51}Cr-release assay or used to produce CTL lines.

RS Virus-Specific CTL Lines. Eight days after *in vitro* restimulation of PBMCs with RS virus, effectors were distributed at 5 × 10^5 cells per well in a 24-well plate (Costar), in 1.5 ml of RPMI/10. To each well was added 1–5 × 10^5 autologous irradiated RS.BCL cells and 0.5 ml of supernatant from MLA144, a gibbon cell line that constitutively secretes interleukin 2 (20). The lines were fed with fresh RPMI/10 and MLA144 supernatant three times a week, and fresh irradiated autologous RS.BCL cells were added (3–5 × 10^5 per well) once a week; the cells were split 1:2 when they reached confluence.

Target Cells Used in CTL Assay. Acutely infected targets were either PBMCs that had been stimulated for 48 hr with phytohemagglutinin (Flow Laboratories) or EBV-transformed BCL cells. Target cells were incubated with 150–300 μCi of ^{51}Cr and simultaneously infected with RS virus at 0.1–1 pfu per cell, for 1 hr at 37°C, then suspended in RPMI/10, incubated for a further 3 hr, and washed three times before use in the CTL assay. Persistently infected targets (RS.BCL cells) were labeled with ^{51}Cr as above, washed three times, and used immediately in a CTL assay. Uninfected BCL cells or phytohemagglutinin-stimulated blasts were used as control targets.

CTL Assay. A standard 5-hr ^{51}Cr-release assay was used, modeled on that used in the mouse system (11). Percent specific lysis was calculated as described (11). Background release of ^{51}Cr ranged from 9% to 18% of the total when CTL lines were used and from 9% to 27% when bulk T-cell cultures were used as effectors.

RESULTS

Human Anti-RS Virus CTL Response. *Bulk cultures of T cells.* Fig. 1 shows that CTLs induced by a single *in vitro* restimulation with RS or influenza virus are specific for the inducing virus. The extent of lysis is low compared with that seen at equivalent effector/target ratios in the mouse secondary anti-RS virus T-cell response (11) and compared with the lysis caused by restimulated CTL lines (Figs. 2 and 3) but is similar to the typical secondary anti-influenza human CTL response seen in the same experiment (Fig. 1).

Both BCL cells and phytohemagglutinin-stimulated PBMCs served as target cells for the CTLs. At an effector/target ratio of 40 or 50:1, the mean lysis of RS virus-infected autologous target cells was 15 ± 2.1% (lysis of uninfected autologous targets was 3.5 ± 3.1%) (mean ± SEM; n = 5 individuals).

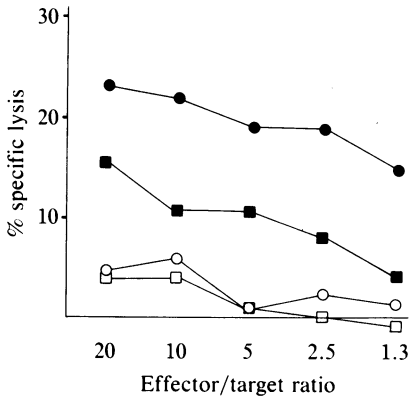

FIG. 1. Restimulation of PBMCs *in vitro* produces CTLs specific for the homologous virus. PBMCs from one adult were restimulated with influenza A/X-31 (●, ○) or RS virus (A2 strain) (■, □). Autologous BCL cells, infected with influenza A/X-31 (●) or RS virus (A2) (■) or uninfected (open symbols), were used as targets in the CTL assay.

Only a low proportion of PBMCs (15 ± 5%) were infected after a 3-hr incubation with RS virus, as assessed by direct immunofluorescence. A very similar result was obtained by Domurat *et al.* (21). This proportion was not materially increased either by incubation for up to 3 days or by treatment with antisera to human α and γ interferons (data not shown). The persistently RS virus-infected BCL cells (see below) served as more sensitive target cells for CTLs.

CTL lines: HLA class I restriction, phenotype, and virus specificity. CTL lines were derived from two individuals, who share no HLA alleles, by restimulation of PBMCs with autologous BCL cells persistently infected with RS virus. Fig. 2 shows that each CTL line lysed only its autologous RS.BCL target but not the uninfected BCL cells. Fig. 3 demonstrates that each CTL line lysed RS.BCL cells sharing only class I alleles; but the persistently infected target line FGRS1, which shares only HLA-DR7 (class II MHC antigen) with the MG.CTL line, was not lysed. These data are consistent with class I MHC restriction of CTL-mediated cytolysis. Infection of FGRS1 was confirmed by direct immunofluorescence, using a mixture of fluorescein isothiocyanate-conjugated anti-N and anti-F mAbs (Imagen, from Boots-Celltech Diagnostics, Slough, Berkshire, U.K.) (results not shown).

Fluorocytometric analysis of indirectly stained cells showed that the MG.CTL and IH.CTL lines contained 96% and 99% $CD8^+$ cells, respectively (control cells stained only with fluorescein isothiocyanate-conjugated anti-mouse immunoglobulin, <0.7%). This result is also consistent with class I HLA restriction of these cell lines (22).

Inhibition of lysis by CD3 and CD8 mAbs. The presence of CD3 or CD8 mAbs (final dilution of mouse ascites 1:200) during the CTL assay inhibited the percent lysis of autologous RS.BCL targets by the IH.CTL line by 44 ± 6% (CD3) and 38 ± 14% (CD8) (mean ± SEM; n = 3 experiments). These figures are comparable with those obtained with other human CTL lines (23).

Persistent RS Virus Infection of BCLs. The persistently RS virus-infected BCL (RS.BCL) cells were convenient both as CTL targets and, after irradiation, to restimulate CTL lines. The RS.BCL cells were specifically lysed by bulk cultures of T cells restimulated with RS virus: the percent specific lysis of the MGRS1 line 6 days after restimulation was 37% (uninfected BCL, 11%) at an effector/target ratio of 72:1.

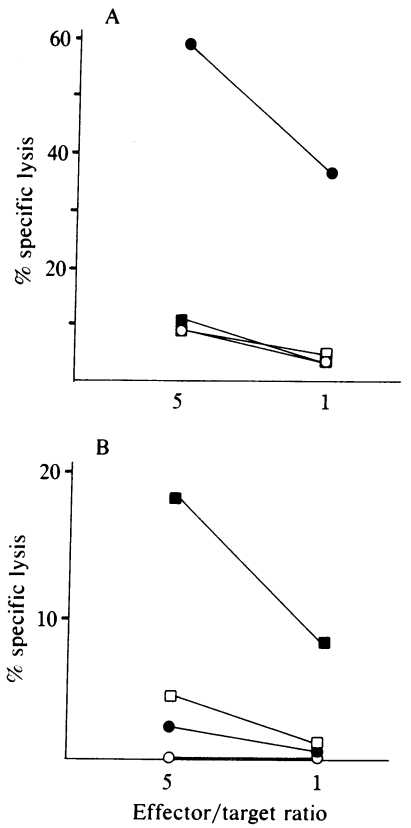

FIG. 2. Self-restriction of RS virus-specific human CTL lines. (*A*) The CTL line from donor MG (MG.CTL) lysed its autologous persistently RS virus-infected BCL (MGRS1; ●) but did not lyse either uninfected autologous MG.BCL cells (○) or allogeneic (donor IH) BCLs, whether uninfected (□) or persistently infected with RS virus (■). (*B*) Conversely, the IH.CTL line lysed the autologous persistently RS virus-infected BCL (■) but not the uninfected autologous BCL (□) nor allogeneic (MG) BCLs, whether uninfected (○) or persistently infected with RS virus (●).

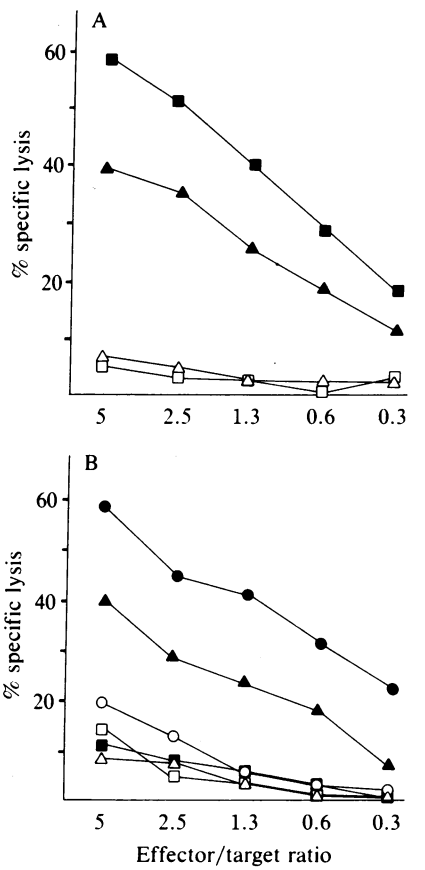

FIG. 3. Restriction of RS virus-specific CTL response by class I alleles of HLA. (*A*) The IH.CTL line lysed both its autologous persistently RS virus-infected BCL (■) and another persistently RS virus-infected BCL (PCRS1) (▲), from an individual (PC) who shares only HLA-A3 and HLA-B15 with IH. Uninfected BCLs from neither individual (□, △) were lysed. (*B*) The MG.CTL line lysed both its autologous persistently RS virus-infected BCL (●), and the PCRS1 line (▲) (as in *A*); PC shares only HLA-B37 with MG. Uninfected BCLs from either MG (○) or PC (△) were not lysed, nor were uninfected or persistently RS virus-infected BCLs from another individual, FG (□, ■), who shares only HLA-DR7 with the effector line, MG.CTL.

Evidence for persistent RS virus infection in RS.BCL cells is presented below.

RS viral surface glycoprotein expression. Table 1 shows that ≈50% of cells expressed each surface glycoprotein of RS virus (F and G); this proportion was unaltered by 3 days of incubation with anti-interferon antisera. Thirty-four percent of MGRS1 and 28% of IHRS1 cells expressed RS virus N protein (uninfected control cells, 0%), as detected by indirect immunofluorescence on acetone-fixed cells.

RS viral antigen synthesis. That the persistently infected cells continued to synthesize viral proteins was confirmed by immunoprecipitation of RS virus N protein (metabolically labeled with [^{35}S]methionine), using two anti-N mAbs (Fig. 4).

Recovery of infectious RS virus from persistently infected cultures. Culture supernatant from the IHRS1 cells was incubated for 36 hr with monolayers of HEp-2 cells, which are permissive for RS virus replication. Since the incubation was continued for longer than the mean RS virus replication time (20 hr), an accurate estimate of the virus titer in the supernatant cannot be made, but the titer cannot exceed the estimate from this assay of 3000 infectious focus-forming units per ml.

Electron microscopy. Fig. 5 shows four transmission electron micrographs of the persistently infected IHRS1 cells, taken 5 months after initial infection. The principal abnormalities are as follows. (*i*) At the surface of some RS.BCL cells (Fig. 5*A*), abundant villous projections can be seen. At higher magnification (Fig. 5*B*), these are clearly identifiable as budding filamentous RS virus (24, 25). (*ii*) The nuclei of some persistently infected cells, apparently those that are actively budding virus, are highly lobulated (Fig. 5*A*). (*iii*) Two types of cytoplasmic inclusions are visible (Fig. 5 *A*, *C*, and *D*), as described by Armstrong *et al.* (26) in HeLa cells acutely infected with RS virus; however, none of these abnormalities was seen in BCL cells after short-term (overnight) infection with RS virus (data not shown).

Table 1. Fluorocytometric analysis of RS virus surface glycoprotein expression on persistently RS virus-infected, EBV-transformed BCLs with or without anti-interferon (IFN) antisera

Cell line	Days post-infection	Anti-IFN	% positive cells		
			Control	Anti-F	Anti-G
MGRS1	120	−	1.4	58	61
		+	1.3	44	55
IHRS1	80	−	5.2	53	48
		+	5.7	58	50

Cultures were passaged as usual (see *Materials and Methods*), and anti-IFN antisera were added to half of each culture for 3 days before indirect immunofluorescence assay using anti-F or anti-G mAb as first antibody or no first antibody (control).

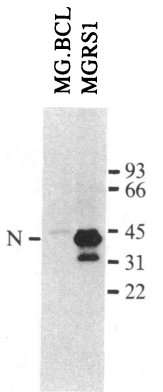

FIG. 4. NaDodSO$_4$/polyacrylamide gel electrophoresis of RS virus N protein, immunoprecipitated with anti-N mAbs and protein A-Sepharose. MG.BCL; uninfected EBV-transformed BCL. MGRS1, the same BCL but persistently infected with RS virus (A2 strain). Proteins were metabolically labeled with [^{35}S]methionine. The N protein is represented by the band at apparent M_r 42,000. The band at 45,000 in each lane represents actin; the band at 32,000 in the MGRS1 lane probably represents RS virus phosphoprotein, coprecipitated from the cell lysate in undissociated nucleocapsids. Markers at right ($M_r \times 10^{-3}$) show positions of standard proteins run in parallel.

DISCUSSION

Virus-Specific CTLs in the Human. The results presented here demonstrate the existence of typical CD8$^+$ CTLs (27) that are specific for RS virus in humans and suggest that these cells are HLA class I-restricted. Similar HLA class I-restricted human CTLs have been produced that are specific for influenza (28), EBV (13), cytomegalovirus (29), and mumps (30); CTL lines specific for human adult T-cell leukemia virus were probably HLA-restricted (31, 32). Measles-specific CTLs in the human are predominantly HLA class II-restricted, CD4$^+$ cells (22), as are a minority of EBV-specific human CTL clones (33).

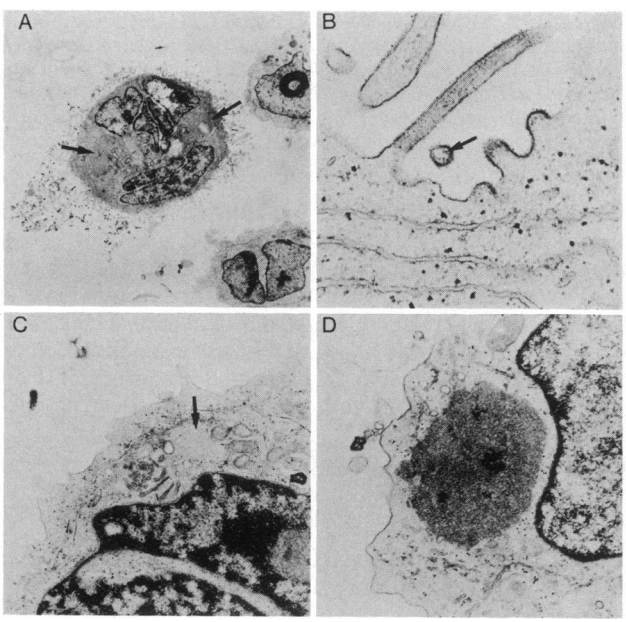

FIG. 5. Transmission electron microscopy of RS.BCL cells. Cells were fixed in 5% glutaraldehyde, postfixed in OsO$_4$, and stained with uranyl acetate and lead citrate. (*A*) Note villous projections from the cell membrane, the abnormal lobular nucleus, and large cytoplasmic inclusions (arrows). (×955.) (*B*) Cell surface, showing budding RS virions. The RS nucleocapsid can be seen in the cross-section of an RS virion (arrow). (×18,920.) (*C*) Filamentous inclusion body (arrow). (×6840.) (*D*) Granular inclusion body. (×6840.)

The importance of cell-mediated immunity in protection against RS virus infections in the human is suggested by clinical observations (e.g., ref. 9) on patients with severe immunodeficiency syndromes (see Introduction). Virus-specific CTL activity in the human has been shown to correlate with recovery from certain virus infections (29, 34); this remains to be tested in RS virus infections.

The pathogenesis of RS virus-induced bronchiolitis or pneumonia is still not understood (35); Kim *et al.* (36) suggested that a cell-mediated response might be actively detrimental. But clear evidence on the role of cell-mediated immunity in both the pathogenesis of the severe disease, and in protection against it, has awaited more precise definition of the cell types involved in each case.

Vaccinia virus recombinants have been used to investigate the viral antigens recognized by the RS virus-specific CTL lines described here. The results (C.R.M.B., unpublished; ref. 50) indicate that the viral nucleoprotein (N) and fusion protein (F) were each recognized by the CTLs, but no evidence for recognition of the major viral glycoprotein G or of the small RS virus structural protein 1A was obtained.

Persistent Infections with RS Virus. Persistent infections with RS virus have been produced *in vitro* with nonlymphoid cells of the human (37), monkey (38), and mouse (39), but we know of no previous report of a persistent infection with RS virus in a cell of lymphoid origin. The low level of infectious RS virus produced by these cells (3000 infectious focus-forming units per ml) is similar to that found by Fernie *et al.* (39) to be produced by cultures of mouse fibroblasts persistently infected with RS virus (1000 pfu per ml). We have not established whether RS virus is also able to infect T cells.

Indirect immunofluorescence staining of RS.BCL cells, using an anti-N protein mAb (Mab15) as first reagent (data not shown) suggests that the large granular cytoplasmic inclusions seen on electron microscopy contain RS virus N protein; viral nucleocapsids accumulate in the cytoplasm of human B-cell lines persistently infected with measles virus (40), and N protein is synthesized in large quantities by the RS.BCLs (Fig. 2).

Persistence of a virus in cell culture may be caused by interferon, by mutations in the virus, or by changes in both virus and host cell (41–43); usually, more than one factor is responsible for persistence. In the present study, interferon did not appear to be a factor in maintaining viral persistence, unlike a persistent measles virus infection of B cells (44). The EBV transformation may play a crucial role in enabling RS virus to persist, as it does in vesicular stomatitis virus persistence in lymphocytes (45).

Domurat *et al.* (21) obtained evidence of RS virus infection of lymphocytes *in vivo* during naturally acquired RS virus disease in human infants. Goswami *et al.* (46) have shown that paramyxoviruses may persist in human bone marrow. Putting these observations together, one may conceive that RS virus causes a persistent infection of lymphocytes, or other bone marrow-derived cells, *in vivo*. Indeed, there is evidence to associate such an infection with Paget disease of bone (e.g., ref. 47). Whether RS virus infection impairs the immune competence of lymphocytes, as measles virus does (48), remains speculative, however.

We thank Professor P. J. Morris, Dr. N. Carter, Dr. A. Ting, and C. Taylor of the Nuffield Department of Surgery for tissue typing and flow cytometry and Dr. D. J. P. Ferguson of the Department of Histopathology for electron microscopy. C.R.M.B. is a Training Fellow of the Medical Research Council (U.K.). The work was supported by a Programme Grant from the Medical Research Council.

1. Kim, H. W., Arrobio, J. O., Brandt, C. D., Jeffries, B. C., Pyles, G., Reid, J. L., Chanock, R. M. & Parrott, R. H. (1973) *Am. J. Epidemiol.* **98,** 216–255.
2. Kim, H. W., Canchola, J. G., Brandt, C. D., Pyles, G., Chanock, R. M., Jensen, K. & Parrott, R. H. (1969) *Am. J. Epidemiol.* **89,** 422–434.
3. Karzon, D. T. (1983) in *Human Immunity to Viruses*, ed. Ennis, F. A. (Academic, New York), pp. 111–130.
4. Walsh, E. E., Schlesinger, J. J. & Brandriss, M. W. (1984) *J. Gen. Virol.* **65,** 761–767.
5. Prince, G. A., Horswood, R. L. & Chanock, R. M. (1985) *J. Virol.* **55,** 517–520.
6. Taylor, G., Stott, E. J., Bew, M., Fernie, B. F., Cote, P. J., Collins, A. P., Hughes, M. & Jebbett, J. (1984) *Immunology* **52,** 137–142.
7. Parrott, R. H., Kim, H. W., Arrobio, J. O., Hodes, D. S., Murphy, B. R., Brandt, C. D., Camargo, E. & Chanock, R. M. (1973) *Am. J. Epidemiol.* **98,** 289–300.
8. Prince, G. A., Horswood, R. L., Camargo, E., Koenig, D. & Chanock, R. M. (1983) *Infect. Immun.* **42,** 81–87.
9. Fishaut, M., Tubergen, D. & McIntosh, K. (1980) *J. Pediatr.* **96,** 179–186.
10. Scott, R., Pullan, C. R., Scott, M. & McQuillin, J. (1984) *J. Med. Virol.* **13,** 105–114.
11. Bangham, C. R. M., Cannon, M. J., Karzon, D. T. & Askonas, B. A. (1985) *J. Virol.* **56,** 55–59.
12. Bangham, C. R. M. & Askonas, B. A. (1986) *J. Gen. Virol.* **67,** 623–629.
13. Moss, D. J., Wallace, L. E., Rickinson, A. B. & Epstein, M. A. (1981) *Eur. J. Immunol.* **11,** 686–693.
14. Routledge, E. G., McQuillin, J., Samson, A. C. R. & Toms, G. L. (1985) *J. Med. Virol.* **15,** 305–320.
15. Cote, P. J., Fernie, B. F., Ford, E. C., Shih, J. W. & Gerin, J. L. (1981) *J. Virol. Methods* **3,** 137–147.
16. Beverley, P. C. L. & Callard, R. E. (1981) *Eur. J. Immunol.* **11,** 329–334.
17. Evans, R. L., Wall, D. W., Platsoucas, C. D., Siegal, F. P., Fikrig, S. M., Testa, C. M. & Good, R. A. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 544–548.
18. Malissen, B., Rebai, N., Liabeuf, A. & Mawas, C. (1982) *Eur. J. Immunol.* **12,** 739–747.
19. Townsend, A. R. M., Gotch, F. M. & Davey, J. (1985) *Cell* **42,** 457–467.
20. Rabin, H., Hopkins, R. F., Ruscetti, F. W., Neubauer, R. H., Brown, R. L. & Kawakami, T. G. (1981) *J. Immunol.* **127,** 1852–1856.
21. Domurat, F., Roberts, N. J., Walsh, E. E. & Dagan, R. (1985) *J. Infect. Dis.* **152,** 895–902.
22. Jacobson, S., Richert, J. R., Biddison, W. E., Satinsky, A., Hartzman, R. J. & McFarland, H. F. (1984) *J. Immunol.* **133,** 754–757.
23. Dongworth, D. W. & McMichael, A. J. (1984) *Br. Med. Bull.* **40,** 254–261.
24. Bächi, T. & Howe, C. (1973) *J. Virol.* **12,** 1173–1180.
25. Parry, J. E., Shirodaria, P. V. & Pringle, C. R. (1979) *J. Gen. Virol.* **44,** 479–491.
26. Armstrong, J. A., Pereira, H. G. & Valentine, R. C. (1962) *Nature (London)* **196,** 1179–1181.
27. Zinkernagel, R. M. & Doherty, P. C. (1979) *Adv. Immunol.* **27,** 51–177.
28. McMichael, A. J. & Askonas, B. A. (1978) *Eur. J. Immunol.* **8,** 705–711.
29. Quinnan, G. V., Kirmani, N., Esber, E., Saral, R., Manischewitz, J. F., Rodgers, J. L., Rook, A. H., Santos, G. W. & Burns, W. H. (1981) *J. Immunol.* **126,** 2036–2041.
30. Kreth, H. W., Kress, L., Kress, H. G., Ott, H. F. & Eckert, G. (1982) *J. Immunol.* **128,** 2411–2415.
31. Kannagi, M., Sugamura, K., Sato, H., Okochi, K., Uchino, H. & Yorio, H. (1983) *J. Immunol.* **130,** 2942–2946.
32. Kannagi, M., Sugamura, K., Kinoshita, K.-I., Uchino, H. & Hinuma, Y. (1984) *J. Immunol.* **133,** 1037–1041.
33. Meuer, S. C., Hodgdon, J. C., Cooper, D. A., Hussey, R. E., Fitzgerald, K. A., Schlossman, S. F. & Reinherz, E. L. (1983) *J. Immunol.* **131,** 186–190.
34. McMichael, A. J., Gotch, F. M., Noble, G. R. & Beare, P. A. S. (1983) *N. Engl. J. Med.* **309,** 13–17.
35. McIntosh, K. & Fishaut, J. M. (1980) *Prog. Med. Virol.* **26,** 94–118.
36. Kim, H. W., Keikin, S. L. & Arrobio, J. O. (1976) *Pediatr. Res.* **10,** 75–78.
37. Baldridge, P. & Senterfit, L. B. (1967) *Proc. Soc. Exp. Biol. Med.* **151,** 684–688.
38. Pringle, C. R., Shirodaria, P. V., Cash, P., Chiswell, D. J. & Malloy, P. (1978) *J. Virol.* **28,** 199–201.
39. Fernie, B. F., Ford, E. C. & Gerin, J. L. (1981) *Proc. Soc. Exp. Biol. Med.* **167,** 83–86.
40. Joseph, B. S., Lampert, P. W. & Oldstone, M. B. A. (1975) *J. Virol.* **16,** 1638–1649.
41. Mahy, B. W. J. (1985) *Br. Med. Bull.* **41,** 50–55.
42. Ron, D. & Tal, J. (1985) *J. Virol.* **55,** 424–430.
43. Treuhaft, M. W. (1984) in *Nonsegmented Negative Strand Viruses*, eds. Bishop, D. H. L. & Compans, R. W. (Academic, New York), pp. 537–544.
44. Jacobson, S. & McFarland, H. F. (1982) *J. Gen. Virol.* **63,** 351–357.
45. Creager, R. S., Whitaker-Dowling, P., Frey, T. K. & Youngner, J. S. (1982) *Virology* **121,** 414–419.
46. Goswami, K. K. A., Cameron, K. R. & Russell, W. C. (1984) *J. Gen. Virol.* **65,** 1881–1888.
47. Mills, B. G., Singer, F. R., Weiner, L. P., Suffin, S. C., Stabile, E. & Holst, P. (1984) *Clin. Orthop. Rel. Res.* **183,** 303–311.
48. McChesney, M. B., Fujinami, R. S., Lampert, P. W. & Oldstone, M. B. A. (1986) *J. Exp. Med.* **163,** 1331–1336.
49. Hildreth, J. E. K. (1982) *Biochem. J.* **207,** 363–366.
50. Bangham, C. R. M., Openshaw, P. J. O., Ball, L. A., King, A. M. Q., Wertz, G. W. & Askonas, B. A. (1986) *J. Immunol.*, in press.