Arch Virol (1990) 110: 91–101

Archives of Virology

Dengue-2 virus infection of human mononuclear cell lines and establishment of persistent infections

I. Kurane, U. Kontny, J. Janus, and **F. A. Ennis**

Division of Infectious Diseases, Department of Medicine, University of Massachusetts Medical Center, Worcester, Massachusetts, U.S.A.

Accepted September 19, 1989

Summary. Twenty three human mononuclear cell lines including ten myelomonocytic cell lines, eight B cell lines and five T cell lines, were examined to determine whether they could be infected with dengue-2 virus. All the cell lines were infected with dengue-2 virus as determined by immunofluorescent staining and by virus titration of culture supernatant fluids. K562, Jiyoye and Jurkat, respectively, showed the highest percentage of infected cells of these myelomonocytic, B and T cell lines. Antibody to dengue-2 virus at subneutralizing concentrations augmented dengue-2 virus infection of myelomonocytic cell lines, but not of B cell lines or of T cell lines.

Persistent dengue-2 virus infection was established using a myelomonocytic cell line (K562), a B cell line (Raji), and a T cell line (HSB-2). These cell lines maintained a high percentage (more than 70%) of dengue-2 virus antigen-positive cells for at least 25 weeks. Very low titers of infectious dengue-2 virus were detected in the culture supernatant fluids of the persistently infected cells. Dengue-2 virus antigen-positive Raji cell clones were established from persistently-infected Raji cells using limiting dilutions and all of the cells in these clones were dengue-2 virus antigen-positive. These findings demonstrate that a variety of human mononuclear cell lines can be infected with dengue-2 virus and may be useful as models for the analysis of dengue virus-human cell interactions in dengue virus infections.

Introduction

Dengue virus infections are serious health problems in many areas of the world; Southeast Asia, Central and South America [14]. Dengue viruses belong to the family *Flaviviridae*, genus *Flavivirus* and are transmitted to humans by mosquitoes. Dengue virus infections cause two forms of syndromes; dengue fever (DF) and dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS)

[12]. DF is a self-limited febrile disease and a most common type of dengue. In some situations patients infected with dengue virus develop life-threatening complications such as hemorrhagic manifestations and shock, which are called DHF/DSS. The pathogenesis of DF and DHF/DSS has not been elucidated. It is speculated that following feeding by mosquitoes, the virus enter blood stream via lymphatics [30] and peripheral blood mononuclear cells (PBMC), especially monocytes, support virus infections [16]. Thus, study of dengue virus-PBMC interaction is important to understand the pathogenesis of dengue virus infections. Many human mononuclear cell lines are available; therefore, we decided to examine a variety of human mononuclear cell lines to determine whether they could be infected with dengue virus and be useful for future studies. In this paper we attempt to infect 23 human mononuclear cell lines, including ten myelomonocytic cell lines, eight B cell lines and five T cell lines with dengue-2 virus. All the cell lines can be infected with dengue-2 virus. Persistent dengue-2 virus infection is established using K562 (myelomonocytic), Raji (B), and HSB-2 (T) cell lines.

Materials and methods

Cell lines

We used human myelomonocytic cell lines K562 [26], HEL92-1-7 [28], JOSK-I,-K,-M, and -S [31], U937 [34], THP-1 [37], KG-1 [20], and HL-60 [8], human B cell lines Jiyoye [32], ARH-77 [6], IM-9 [10], Raji [9], HS-Sultan [17], CA46 [27], Daudi [18], and Ramos [19], and human T cell lines Jurkat, CEM [11], HSB-2 [1], Molt 3 and Molt 4 [29]. K562, HEL92-1-7, U937, THP-1, KG-1, HL-60, Jiyoye, ARH-77, IM-9, Raji, HS-Sultan, CA46, Daudi, Ramos, CEM, HSB-2, Molt 3, and Molt 4 were purchased from American Tissue Culture Collection (ATCC). JOSK-I, JOSK-K, JOSK-M, and JOSK-S were provided by Dr. Masatsugu Ohta of Jichi Medical School, Japan. CA46, Daudi, and Jurkat were provided by Dr. John Sullivan of University of Massachusetts Medical Center, Worcester, MA, U.S.A. All cell lines were maintained in RPMI 1640 medium (Flow Laboratories, McLean, VA, U.S.A.) supplemented with 10% fetal bovine serum (FBS) (GIBCO Laboratories, Grand Island, NY, U.S.A.), penicillin (100 U/ml) and streptomycin (100 μg/ml).

Virus and antibody

Dengue-2 virus, New Guinea C strain, was used for infection. The virus was received from Dr. Walter E. Brandt of the Walter Reed Army Institute of Research, Washington, DC. The virus has been passed in mouse brain and was then propagated in *Aedes albopictus* (mosquito) cells (C6/36) as previously described [22]. The titer of the virus pool used in these experiments was 1×10^7 plaque forming unit (p.f.u.)/ml in Vero cells using previously described methods [22]. Ascitic fluid from mice hyperimmunized with dengue-2 virus was used as a source of anti-dengue-2 virus antibody. This antibody was also supplied by Dr. Brandt. The titer of this antibody was 1,024 as determined by a plaque neutralization test [23]. Hyperimmune ascitic fluid was heated at 56 °C for 30 min to destroy complement activity before use.

Infection of cells with dengue virus

Cells were washed once in RPMI 1640 containing 1% FBS and suspended at a concentration of 2×10^5 cells in 0.1 ml. They were then incubated with dengue-2 virus or with dengue-2

virus antibody complexes at 37 °C for 2 h. These virus-antibody complexes were prepared by adding 10 µl of anti-dengue-2 virus antibody at a dilution of 1 : 200 to 5×10^6 p.f.u. of dengue-2 virus in 0.5 ml and were incubated at 4 °C for 1 h. The multiplicity of infection (m.o.i.) of the virus inoculum was 5 p.f.u. per cell. Cells were washed twice and resuspended at a concentration of 2×10^5 cells per ml in RPMI 1640 containing 10% FBS. Cells were stained for the presence of dengue-2 viral antigen by indirect immunofluorescence (IF) at 24, 48, and 72 h as previously described [21]. The first antibody was the murine hyperimmune ascitic fluid to dengue-2 virus and the second antibody was a fluorescein isothiocyanate (FITC)-conjugated sheep anti-mouse immunoglobulin G antibody (Cappel Laboratories, Malvern, PA, U.S.A.).

Titration of dengue-2 virus

The amount of infectious virus produced by each cell line was determined by plaque assay in Vero cells. Intracellular virus was assayed after three freeze-thaw cycles with cells at concentration of 1×10^6/ml [35, 36]. One-tenth ml of serially 10-fold-diluted culture supernatant fluid was placed on Vero cell monolayers in 24-well plates (Costar, Cambridge, MA, U.S.A.) and incubated at 37 °C for 2 h. The supernatant fluid was then removed, the cells were washed once with MEM containing 2% FBS, and 1 ml 1% agar (Difco Laboratories, Detroit, MI, U.S.A.) containing RPMI 1640, 10% FBS, 0.5% non-essential amino acids, 0.5% essential vitamins and 0.3% DEAE-dextran was used as an overlay. The plates were incubated at 37 °C for 7 days, stained with neutral red and the plaques counted [22].

Establishment of persistent infection

Cells were infected with dengue-2 virus at a m.o.i. of 5 p.f.u./ml in the absence of antibody as described above and were cultured at 2×10^5/ml in RPMI containing 10% FBS. Cells were resuspended at a concentration of 2×10^5/ml every 3 or 4 days. The percent of dengue antigen-positive cells was examined weekly using IF.

Establishment of dengue-2 virus antigen-positive Raji cell clones

Dengue-2 antigen-positive Raji cell clones were established using a limiting dilution technique. Persistently infected Raji cultures, 90% of which had dengue-2 virus antigen, were cultured at 0.5 cell/well (1 cell/2 wells) in 0.2 ml RPMI containing 10% FBS in 96 well flat bottom plates (Costar, Cambridge, MA, U.S.A.). Cells were cultured for 3 weeks. Growing cells were examined for cytoplasmic dengue-2 virus antigen using IF.

Results

Acute infection of human mononuclear cell lines with dengue-2 virus

Ten myelomonocytic cell lines, eight B cell lines, and five T cell lines were used in the experiments. All the cell lines could be infected with dengue-2 virus in the absence of antibody (Table 1). Antibody to dengue-2 virus augmented dengue-2 virus infection of myelomonocytic cell lines determined by IF and virus assays. However, antibody did not augment infection of B or T cell lines. In myelomonocytic cell lines K562, HEL92-1-7, JOSK-I, and JOSK-M cells contained a high percentage of antigen-positive cells, while HL-60, KG-1, and THP-1 contained fewer antigen-positive cells. In B cell lines Jiyoye, ARH-77, and IM-9 contained a high percentage of antigen-positive cells, while Ramos, Daudi and CA46 contained fewer antigen-positive cells. In T cell lines Jurkat and CEM contained a high percentage of antigen-positive cells.

Table 1. Dengue-2 virus infection of human mononuclear cell lines*

Cell line	Antibody**	% dengue-2 antigen-positive cells			Virus titer (p.f.u./ml) at 48 h
		24 h	48 h	72 h	
Myelomonocytic cell lines					
K562	+	90.8	95.9	99.0	5.0×10^6
	−	57.8	76.5	99.0	4.5×10^6
HEL92-1-7	+	35.8	35.4	41.8	2.3×10^5
	−	22.8	26.5	37.2	1.5×10^5
JOSK-I	+	37.5	34.3	18.5	5.0×10^5
	−	7.7	4.1	1.7	5.7×10^4
JOSK-M	+	28.6	23.0	8.4	2.3×10^5
	−	16.0	11.0	3.5	2.3×10^5
JOSK-S	+	16.1	9.5	6.4	1.6×10^5
	−	6.3	3.8	3.4	1.5×10^5
JOSK-K	+	15.1	14.1	3.9	4.0×10^5
	−	3.6	3.7	3.3	1.6×10^5
U937	+	15.7	17.4	4.1	3.8×10^4
	−	0.6	1.3	0.9	4.2×10^3
THP-1	+	7.5	6.8	9.8	1.1×10^5
	−	0.4	0.5	0.5	2.0×10^4
KG-1	+	2.4	1.7	0.9	1.3×10^4
	−	1.4	1.3	0.5	3.0×10^3
HL-60	+	1.7	2.9	1.6	1.4×10^3
	−	0.2	0.4	0.3	3.5×10^2
B cell lines					
Jiyoye	+	82.6	81.4	81.7	4.0×10^4
	−	82.4	86.4	85.0	5.0×10^4
ARH-77	+	28.4	45.7	30.8	5.4×10^5
	−	25.0	30.9	31.4	3.9×10^5
IM-9	+	22.6	22.7	17.2	3.5×10^5
	−	30.3	25.3	13.1	3.1×10^5
Raji	+	10.2	6.6	11.7	8.5×10^3
	−	7.8	9.6	13.7	9.5×10^3
HS-Sultan	+	8.8	6.9	8.3	4.0×10^3
	−	4.8	5.6	7.5	3.0×10^3
CA46	+	1.5	1.5	1.3	4.0×10^3
	−	2.5	2.2	1.0	2.0×10^3

Table 1 continued

Cell line	Antibody**	% dengue-2 antigen-positive cells			Virus titer (p.f.u./ml) at 48 h
		24 h	48 h	72 h	
Daudi	+	1.1	1.1	0.8	1.0×10^2
	−	0.5	1.8	0.8	1.0×10^2
Ramos	+	0.8	0.5	0.4	8.0×10^0
	−	0.5	0.5	0.3	5.0×10^0
T cell lines					
Jurkat	+	50.0	39.2	39.7	6.6×10^4
	−	48.7	44.5	34.5	6.1×10^4
CEM	+	36.3	27.0	21.3	1.3×10^5
	−	34.0	23.4	18.5	1.5×10^5
HSB-2	+	13.8	24.4	81.7	1.0×10^5
	−	19.5	28.5	84.0	3.0×10^5,
Molt 4	+	8.2	16.8	41.7	1.1×10^5
	−	7.6	15.3	44.4	1.7×10^5
Molt 3	+	4.0	12.9	14.0	7.5×10^3
	−	8.1	12.0	21.3	1.3×10^4

* Cells were infected with dengue-2 virus at a m.o.i. of 5 p.f.u./cell in the presence or absence of antibody as described in Materials and methods

** Anti-dengue-2 antibody was used at final dilution of 1 : 10,000. + Presence of antibody, − absence of antibody

Establishment of cell lines persistently infected with dengue-2 virus

We determined the ability of certain cells to become persistently infected with dengue-2 virus. K 562, Raji, and HSB-2 cell lines were infected with dengue-2 virus at m.o.i. of 5 p.f.u./cell in the absence of antibody to dengue-2 virus, and were cultured for 25 weeks. Cells were resuspended at a concentration of 2×10^5/ml twice a week. The percent of dengue-2 virus antigen-positive cells was almost 100% one week after infection of K 562 (Fig. 1) and HSB-2 cells (Fig. 3), and three weeks after infection of Raji cells (Fig. 2). A high percentage (more than 70%) of dengue-2 virus antigen-positive cells was observed for 25 weeks. These results demonstrate that persistent dengue-2 virus infections were readily established in myelomonocytic, B and T cell lines.

We measured dengue-2 virus titers in culture supernatant fluids 22 weeks after the infection. Infectious dengue-2 virus at the titer of 2.6×10^2 p.f.u./ml, 1.8×10^2 p.f.u./ml, and 1.0×10^2 p.f.u./ml were detected in the supernatant fluids of persistently infected K 562, HSB-2, and Raji, respectively. Intracellular

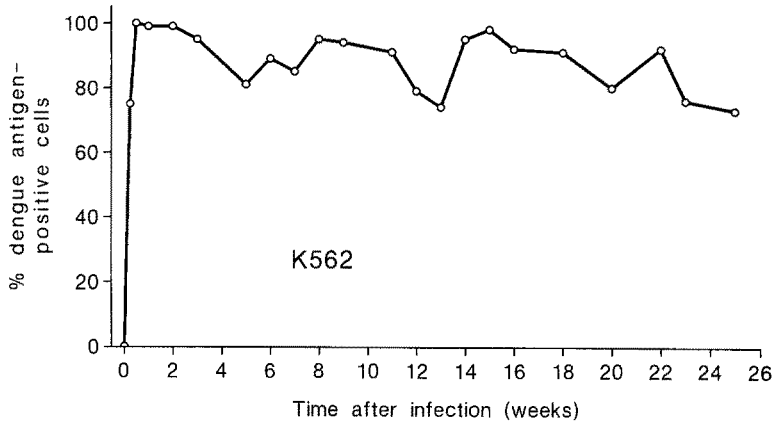

Fig. 1. Persistent infection of K562 cells infected with dengue-2 virus at a m.o.i. of 5 p.f.u./cell. Cells were resuspended at 2×10^5/ml every 3 or 4 days. Percentage of dengue-2 virus antigen-positive cells was determined using IF

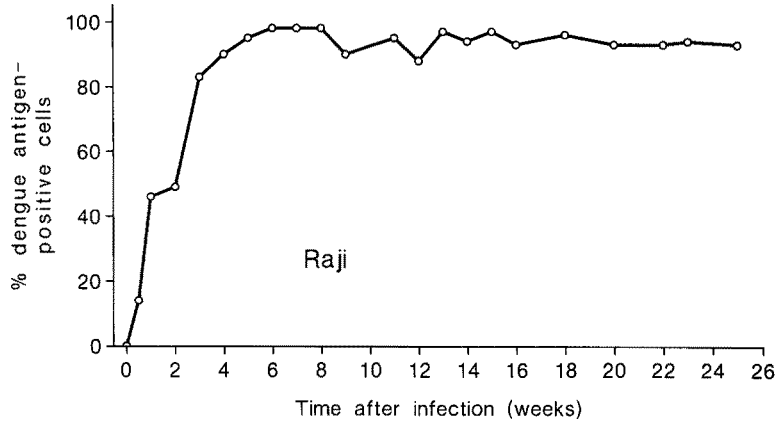

Fig. 2. Persistent infection of Raji cells infected with dengue-2 virus at a m.o.i. of 5 p.f.u./cell. Cells were resuspended at 2×10^5/ml every 3 or 4 days. Percentage of dengue-2 virus antigen-positive cells was determined using IF

dengue virus was then detected after three freeze-thaw cycles of 1×10^6 infected cells. Intracellular virus titers were 2.2×10^2 p.f.u./ml and 4.0×10^1 p.f.u./ml in K562 and HSB-2 cells, respectively. Intracellular virus was not detected (< 10 p.f.u./ml) in persistently infected Raji cells.

Establishment of Raji cell clones persistently infected with dengue-2 virus using a limiting dilution technique

Using a limiting dilution technique, we tried to determine whether a single dengue-2 virus antigen-positive cell can proliferate to produce multiple antigen-positive cells. Persistently infected Raji cells, 90% of which were antigen-pos-

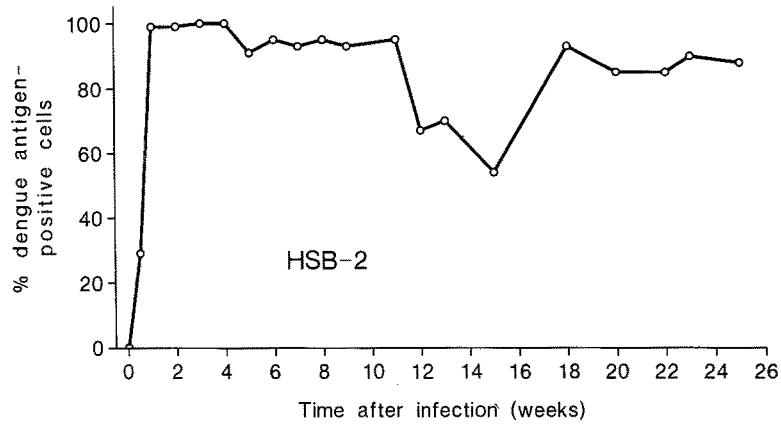

Fig. 3. Persistent infection of HSB-2 cells infected with dengue-2 virus at a m.o.i. of 5 p.f.u./cell. Cells were resuspended at 2×10^5/ml every 3 or 4 days. Percentage of dengue-2 virus antigen-positive cells was determined using IF

itive, were cultured at a concentration of 0.5 cells/well. The clonal lines were examined for dengue-2 virus antigens after 3 weeks. 61 clones were established, 54 (89%) of which contained 100% antigen-positive cells, and seven (11%) of which contained no antigen-positive cells.

Discussion

In this paper dengue-2 virus-human cell interactions have been studied using human mononuclear cell lines. We have demonstrated that all of the 23 human mononuclear cell lines including ten myelomonocytic cell lines, eight B cell lines, and five T cell lines can be infected with dengue-2 virus. It has been reported that human monocytes are the cells that predominantly support infections with dengue viruses [16]. It has also been reported that B blast cells prepared from PBMC and Epstein-Barr virus (EBV)-transformed B lymphoblastoid cell lines could be infected with a dengue virus [35, 36], but that T blast cells prepared from PBMC or a T cell line, Molt-4, could not be infected [36]. In our study we examined five human T cell lines, and all could be infected with dengue-2 virus. A human dengueviruses-specific T cell clone, which was established from the PBMC of a donor who had been previously infected with dengue-3 virus and has the $CD3^+$, $CD4^+$, $CD8^-$ phenotype [24], could be infected with dengue-2 virus (data not presented). Our success in infecting human T cells may be due to the strain of dengue-2 virus or due to the higher m.o.i. we used. We used the New Guinea C strain at a m.o.i. of 5, while Theofilopoulos et al. who reported that T cells could not be infected with dengue-2 virus used the 16681 strain at a m.o.i. of 0.05 [36]. Although most reports have shown that cells infected with dengue virus have monocyte-like morphology in vivo [3, 4],

Scott et al. isolated dengue virus from the non-adherent fraction of PBMC from patients [33]. Our results suggest that B and T cells may support the replication of dengue-2 virus and probably other dengue viruses, especially if the B and T cells are activated as they are in these cell lines.

It has been reported that subneutralizing concentrations of antibody to dengue viruses augment dengue virus infection of Fc_γ receptor-positive cells [15]. This is due to the uptake of virus-antibody complexes by Fc_γ receptor-positive cells, and may contribute to the pathogenesis of severe complications of dengue [7, 13]. In the present study all the myelomonocytic cell lines showed antibody-dependent enhancement of dengue-2 virus infection, whereas we did not observe enhancement using B cell lines or T cell lines. It has been reported that human B cells and some B cell lines have Fc_γRII similar to human monocytes and myelomonocytic cell lines [2]. Using K562 cells, we have found that Fc_γRII mediates antibody-dependent enhancement [R. Littaua et al., submitted for publ.]. However, it has also been reported that Fc_γRII of B cell lines may be somewhat different from Fc_γRII of monocytes and myelomonocytic cell lines, because a monoclonal antibody to Fc_γRII of K562 did not react with FC_γRII of B cell lines, Daudi and Raji [25]. This may explain the absence of antibody-dependent enhancement of dengue-2 virus infection using B cell lines. Studies of antibody-dependent enhancement have been mainly performed using U937 cells [5]. Our results suggest that other myelomonocyte cell lines, such as THP-1 and JOSK-I, are also useful for the study of antibody-dependent enhancement of virus infection with dengue viruses and perhaps other flaviviruses.

In this study we established persistent dengue-2 virus infections in a myelomonocytic cell line (K562), a B cell line (Raji) and a T cell line (HSB-2). Persistent dengue-2 virus infection of Raji cells has been reported [35], and we previously used persistently infected Raji cells as targets in cell killing assays [22] and as inducer of interferon (IFN) from PBMC [23]. The present results show that human myelomonocytic and T cells also support persistent dengue-2 virus infections. The interaction between dengue-2 virus and myelomonocytic, T and B cells and the mechanism of virus persistence are interesting subjects to be elucidated. Raji cell clones were established and the percent of antigen-positive and -negative clones was found to be similar to the frequency of dengue-2 virus antigen-positive and -negative cells in the original culture. All of the cells in these antigen-positive clones continued to contain dengue-2 virus antigen, and all of the cells in the antigen-negative clones continued to be dengue-2 virus antigen-negative. These results suggest that dengue-2 virus antigen-positive cells may proliferate and produce antigen-positive progeny cells.

These experiments demonstrate that dengue-2 virus readily establishes persistent infections in human T, B, and myelomonocytic cell lines in vitro. It is conceivable that persistent infections may occur in vivo and that infected cells may continue to stimulate dengue virus-specific memory T lymphocytes to maintain dengue virus-specific immunity.

Acknowledgement

This work was supported by grants from the U.S. Army Medical Research and Development Command (DAMD 17-86-C-6208), and from the National Institutes of Health (NIH-T32-AI07272). The opinions contained herein are those of the authors and should not be construed as representing the official policies of the Department of Army or the Department of Defense.

References

1. Adams RA, Flowers A, Davis BJ (1968) Direct implantation serial transplantation of human acute lymphoblastic leukemia in hamsters, SB-2. Cancer Res 28: 1121–1125
2. Anderson CL, Looney RJ (1986) Human leukocyte IgG Fc receptors. Immunol Today 7: 164–266
3. Boonpucknavig S, Boonpucknavig V., Bhamarapravati N., Nimmannitya S (1979) Immunofluorescence study of skin rash in patients with dengue hemorrhagic fever. Arch Pathol Lab Med 103: 463–466
4. Boonpucknavig V, Bhamarapravati N, Boonpucknavig S, Futrakul P, Tanpaichitr P (1976) Glomerular changes in dengue hemorrhagic fever. Arch Pathol Lab Med 100: 206–212
5. Brandt WE, McCown JM, Gentry MK, Russell PK (1982) Infection enhancement of dengue type 2 virus in the U-937 human monocyte cell line by antibodies to flavivirus cross-reactive determinants. Infect Immun 36: 1036–1041
6. Burk KH, Drewinko B, Trujillo JM, Ahearn MJ (1978) Establishment of human plasma cell line in vitro. Cancer Res 38: 2508–2513
7. Burke DS, Nisalak A, Johnson DE, Scott RM (1988) A prospective study of dengue infections in Bangkok. Am J Trop Med Hyg 38: 172–180
8. Collins SJ, Gallo RC, Gallagher RE (1977) Continuous growth and differentiation of human myeloid leukemic cells in suspension culture. Nature 270: 347–349
9. Epstein MA, Barr YM (1965) Characteristics and mode of growth of a tissue culture strain (EB1) of human lymphoblasts from Burkitt's lymphoma. J Nat Cancer Inst 34: 231–240
10. Fahey JL, Buell DN, Sox HC (1971) Proliferation and differentiation of lymphoid cells: studies with human lymphoid cells and immunoglobulin synthesis. Ann NY Acad Sci 190: 221–234
11. Foley GE, Lazarus H, Farber S, Uzman BG, Boone BA, McCarthy RE (1965) Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. Cancer 18: 522–529
12. Halstead SB (1980) Immunological parameters of togavirus disease syndrome. In: Schlesinger RW (ed) The Togaviruses: biology, structure, replication. Academic Press, New York, p 107
13. Halstead SB (1981) The pathogenesis of dengue: molecular epidemiology in infectious disease. Am J Epidemiol 114: 632–648
14. Halstead SB (1981) Dengue hemorrhagic fever—a public health problem and a field for research. Bull WHO 58: 1–21
15. Halstead SB, O'Rourke EJ (1977) Dengue virus and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. J Exp Med 146: 201–217
16. Halstead SB, O'Rourke EJ, Allison AC (1977) Dengue virus and mononuclear phagocytes. II. Identity of blood and tissue leukocytes supporting in vitro infection. J Exp Med 146: 218–229
17. Harris NS (1974) Plasma cell surface antigen on human plasma lymphocytes. Nature 250: 507–509

18. Klein E, Klein G, Nadkarni S, Nadkarni JJ, Wigzell H, Clifford P (1968) Surface IgM-kappa specificity on a Burkitt lymphoma cell in vivo and in derived culture lines. Cancer Res 28: 1300–1310
19. Klein G, Giovanella B, Westman A, Stehlin JS, Mumford D (1975) An EBV-genome-negative cell line established from an American Burkitt lymphoma; receptor characteristics, EBV infectivity and permanent conversion into EBV-positive sublines by in vitro infection. Intervirology 5: 319–334
20. Koeffler HP, Golde DW (1978) Acute myelogenous leukemia: a human cell line responsive to colony-stimulating activity. Science 200: 1153–1154
21. Kurane I, Ennis FA (1987) Induction of interferon from human lymphocytes by autologous, dengue virus-infected monocytes. J Exp Med 166: 999–1010
22. Kurane I, Hebblewaite D, Brandt W, Ennis FA (1984) Lysis of dengue-infected cells by natural cell-mediated cytotoxicity and antibody-dependent cell-mediated cytotoxicity. J Virol 52: 223–230
23. Kurane I, Meager A, Ennis FA (1986) Induction of interferon alpha and gamma from human lymphocytes by dengue virus-infected cells. J Gen Virol 67: 1653–1661
24. Kurane I, Meager A, Ennis FA (1989) Dengue virus-specific human T cell clones: serotype crossreactive proliferation, interferon gamma production, and cytotoxic activity. J Exp Med 170: 763–775
25. Looney RJ, Abraham GN, Anderson CL (1986) Human monocytes and U937 cells bear two distinct Fc receptors for IgG. J Immunol 136: 1641–1647
26. Lozzio CB, Lozzio BB (1975) Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. Blood 45: 321–334
27. Magrath IT, Pizzo PA, Whang-Peng J, Douglass EC, Alabaster O, Gerber P, Freeman CB, Novikovs L (1980) Characterization of lymphoma-derived cell lines: comparison of cell lines positive and negative for Epstein-Barr Virus nuclear antigen. I. Physical, cytogenetic, and growth characteristics. J Nat Cancer Inst 64: 465–473
28. Martin P, Papayannopoulou T (1982) HEL cells: a new human erythroleukemia cell line with spontaneous and induced globin expression. Science 216: 1233–1235
29. Minowada J, Ohnuma T, Moore GE (1972) Rosette-forming human lymphoid cell lines. I. Establishment and evidence for origin of thymus-derived lymphocytes. J Nat Cancer Inst 49: 891–895
30. Monath TP (1985) Flaviviruses. In: Fields BN, Knipe DM, Chanock RM, Roizman B, Shope R (eds) Virology. Raven Press, New York, p 955
31. Ohta M, Furukawa Y, Ide C, Akiyama N, Utakoji T, Miura Y, Saito M (1986) Establishment and characterization of four human monocytoid leukemia cell lines (JOSK-I, -S, -M, and -K) with capabilities of monocyte-macrophage lineage differentiation and constitutive production of interleukin 1. Blood 46: 3067–3074
32. Pulvertaft RJV (1965) A study of malignant tumours in Nigeria by short-term tissue culture. J Clin Pathol 18: 261–273
33. Scott RM, Nisalak A, Cheamudon U, Seridhoranakul S, Nimmannitya S (1980) Isolation of dengue viruses from peripheral blood leukocytes of patients with hemorrhagic fever. J Infect Dis 141: 1–6
34. Sundstrom C, Nilsson K (1976) Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int J Cancer 17: 565–577
35. Sung JS, Diwan AR, Falkler WA Jr, Yang H-Y, Halstead SB (1975) Dengue carrier culture and antigen production in human lymphoblastoid lines. Intervirology 5: 137–149
36. Theofilopoulos AN, Brandt WE, Russell PK, Dixon FT (1976) Replication of dengue-2 virus in cultured human lymphoblastoid cells and subpopulations of human peripheral leukocytes. J Immunol 117: 953–961

37. Tsuchiya S, Yamabe M, Yamaguchi Y, Kobayashi Y, Konno T, Tada K (1980) Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). Int J Cancer 26: 171–176

Authors' address: F. A. Ennis, M.D., Division of Infectious Diseases, Department of Medicine, University of Massachusetts Medical Center, Worcester, MA 01655, U.S.A.

Received July 26, 1989