

Antigen-Specific Expansion of Cytotoxic T Lymphocytes in Acute Measles Virus Infection

JUTHATHIP MONGKOLSAPAYA,¹ ASSAN JAYE,² MARGARET F. C. CALLAN,¹
ALBERT F. MAGNUSEN,² ANDREW J. McMICHAEL,^{1*}
AND HILTON C. WHITTLE²

Molecular Immunology Group, Institute of Molecular Medicine, The John Radcliffe, Headington, Oxford OX3 9DS, United Kingdom,¹ and MRC Laboratories, Banjul, Fajara, The Gambia²

Received 3 August 1998/Accepted 23 September 1998

Skewing of the T-cell receptor repertoire of CD8⁺ T cells has been shown in some persistent infections with viruses, such as human immunodeficiency virus, simian immunodeficiency virus, and Epstein-Barr virus. We have demonstrated that similar distortions also occur in nonpersistent measles virus infection. In addition, two of four children immunized with live, attenuated measles virus showed larger and more persistent CD8⁺ T-cell expansions than their naturally infected counterparts. The expanded lymphocyte populations were monoclonal or oligoclonal and lysed target cells infected with recombinant vaccinia virus expressing measles virus protein. These results demonstrate that the expansions of CD8⁺ T lymphocytes are antigen driven.

Measles virus (MV) is a negative-strand RNA virus. The clinical symptoms caused by MV infection appear between 2 and 3 weeks after infection. The appearance of the rash is a sign of the peaking immune response and is associated with clearance of the virus (17). Although now well controlled by vaccination programs in developed countries, measles is still a major problem in sub-Saharan Africa (14, 15). Children below the age of 1 year are particularly at risk and are hard to protect with the current vaccine (30). In young children, MV causes around one million deaths per annum and there is also a large morbidity. Secondary infection by other agents is common (2, 20), while malnutrition may increase the risk of this complication (1, 32). Such secondary infections are believed to largely result from immunosuppression by MV infection which can persist for several weeks (33). The effect was first described as a delayed type hypersensitivity defect in MV-infected patients (37). Proliferation of lymphocytes in response to mitogen *in vitro* is also reduced after MV infection (16, 33, 39, 42).

While the humoral immune response is important in protecting against reinfection, the cellular response, especially the CD8⁺ T-cell response, is important in the clearance of established MV infection (3, 18, 36). Some primary infections with persistent viruses, such as human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV), and Epstein-Barr virus (EBV), stimulate a very strong CD8⁺ T-cell response, with development of large clonal and oligoclonal expansions of these cells (5, 7, 25, 40). Such expansions may comprise up to 40% of CD8⁺ T cells and thus cause distortions of the T-cell receptor (TCR) repertoire. Clonally expanded populations can persist for many months after primary infection with these persistent viruses. While indirect evidence suggests that these T-cell expansions are antigen driven, little is known about their functional capacity, such as cytotoxicity.

In this study, we wished to determine whether similar distortions in the T-cell repertoire occur in MV infection. We therefore studied the TCR repertoire in peripheral blood lymphocytes of children with acute MV infection and of healthy

Gambian infants given the standard live, attenuated MV vaccine. We found marked distortions of the T-cell repertoires in the majority of infected patients and in two of the four vaccinees. These expanded T lymphocytes have cytotoxic activity and are likely therefore to play a major role in the clearance of infected cells.

MATERIALS AND METHODS

Patients and controls. Nineteen patients with acute MV infection from The Gambia were studied. The patients were aged from 6 months to 9 years, with a mean of 2.8 years. Categorization of disease severity was based on previously described criteria (21). Five showed severe and 10 showed moderate symptoms; the rest had mild disease. The study also included four children who were vaccinated with the attenuated Edmonston strain of MV at the age of 9 months. Patients were bled within a week of the onset of rash, and vaccinees were sampled 2 to 4 weeks after vaccination. Ten Gambian children, with a mean age of 8.1 years, recovering from malaria infection at least 4 months, were included as controls. Approval for this study was given by the Gambian Government/MRC Ethics Committee.

Isolation and fractionation of lymphocyte preparations. Peripheral blood mononuclear cells (PBMC) were isolated from whole blood by Ficoll-Hypaque density gradient centrifugation. The cells were cryopreserved until tested. In the clonality expansion study, CD4⁺ cells were depleted from PBMC by anti-CD4-conjugated Dynabeads (DynaL UK Ltd.).

Flow cytometry analysis for T-cell repertoire. Two-color staining was carried out as described previously (28). PBMC were stained with a panel of monoclonal antibodies (MAb) specific for the β -chain variable region of the TCR ($V\beta$) by using a second-layer rabbit anti-mouse antibody directly conjugated to fluorescein isothiocyanate (Dako Ltd.). The anti-CD8 MAb was directly conjugated to phycoerythrin (Dako Ltd.). The panel of anti-human TCR $V\beta$ -region MAb is as follows: B237.2 (BV1), E2.2E7.2 (BV2), LE89 (BV3), 30/3D6 (BV5S2/S3), OT145 (BV6S7), 3G5D5 (BV7S1), JR2 (BV8), MKB1P.2-10 (BV9), S511 (BV12), H131 (BV13S1), H132 (BV13S2), C1 (BV17), ELL1.4 (BV20), 1G125 (BV21S3), and HUT78#7 (BV23). These antibodies were part of the human TCR MAb workshop. Cells were analyzed on a Becton-Dickinson FACScan using CELLQUEST software. CD8⁺ T lymphocytes within an individual $V\beta$ family were considered to have attained significant expansion when the percent staining was higher than three standard deviations above the mean staining of that $V\beta$ family in the control population.

Molecular cloning and sequencing of TCR beta chains. RNA was extracted from CD4-depleted T cells by using TRIreagent (Sigma Chemical Co.) and used in first-strand cDNA synthesis with CB14 primer, CTCAGCTCCACGTG as described previously (40). The cDNA was used as a template in PCRs using 3' CB-R primer (ATACTGGAGTTCGACCTTCTGTATGGCTCAAACAC) and 5' BV primer. The 5' primers were BV7, ATAAGAATGCGGCCGCGTTTGTCTACAGCTATGAGAACTCT; BV8, TTCTAGAAGCGGCCGACAGTTCCGATAGATGATTACAG; and BV17, ATAAGAATGCGGCCGACAGCGTCTCTCGGGAGA. The PCR conditions were as previously described (5). The PCR products were gel purified using the Wizard PCR purification kit (Promega). The purified product was cloned in a pMOSBLUE T-vector (Amersham

* Corresponding author. Mailing address: Molecular Immunology Group, Institute of Molecular Medicine, The John Radcliffe, Headington, Oxford OX3 9DS, United Kingdom. Phone: 44 1865 222336. Fax: 44 1865 222502. E-mail: andrew.mcmichael@ndm.ox.ac.uk.

Life Sciences). Plasmid DNA was extracted by the DNA purification system (Promega). Double-stranded DNA sequencing was performed with T7 DNA polymerase (Pharmacia) and M13 -20 primer (GTAAAACGACGGCCAGT).

Depletion of expanded T cells. PBMC were cultured overnight at 37°C in the presence of 10 U of interleukin-2/ml of RPMI 1640 plus 10% fetal calf serum (R10). Cells were counted for live cells and split into two groups. One group was kept as untreated cells. The other was incubated with antibodies against the expanded V β TCR for 1 h at 4°C. After washing, goat anti-mouse immunoglobulin G-coated Dynabeads (Dyna) were added to the cells and rotated for 1 h at 4°C and then removed using a magnet. Unbound cells were tested for cytotoxic T-lymphocyte (CTL) activity.

CTL activity. CTL activity was assayed as described previously (18). Briefly, autologous EBV-transformed B cells, target cells, were infected with vaccinia virus recombinants expressing the measles virus fusion protein (F), hemagglutinin (H), or nucleoprotein (NP) at a multiplicity of infection of 5 for 2 h at 37°C. Cells were then washed and left at 37°C in R10 for 16 h before labelling them with ⁵¹chromium. An effector-to-target ratio of 20 to 1 was used in a 4-h ⁵¹chromium release assay.

RESULTS

The CD8⁺ T-cell repertoire is distorted during acute MV infection. By staining PBMC with a panel of 15 MAbs specific for the TCR V β chain, we found expanded populations of CD8⁺ T cells expressing particular V β chains (V β expansions) in 16 of 19 children with acute MV infection and 2 of 4 vaccinees (Table 1). Up to four different V β expansions were found in any one individual. The expansions were not confined to particular TCR V β families, and there was no association with HLA class I (Table 1). There was no correlation between severity of the disease and the number or size of the expansions (data not shown). Follow-up samples were examined 4 to 9 months after acute infection and 8 to 10.5 months after vaccination. By this time, most of the expansions were reduced in size or were no longer detectable, with only a few V β expansions persisting.

In contrast, the CD8⁺ TCR repertoires of the children recovering from malaria infection were very similar to each other and to those previously recorded in control Caucasian populations (Table 1). They showed no expansions.

CD8⁺ TCR V β expansions are clonal or oligoclonal. To determine whether the expansions observed were derived from one (clonal) or a few (oligoclonal) T-cell clones, we examined the predicted amino acid sequences of the V β chain CDR3 regions (between V β and J β) of the expanded CD8⁺ T cells in three children. In patient M42, 64% of the expanded BV8⁺ CD8⁺ T-cell transcripts were identical, showing that a single clone of T cells accounted for most of this expansion of 16% of CD8⁺ T cells (Table 2). In the follow-up sample taken from this child, the repertoire, as assessed using TCR V β -specific MAbs, had returned to normal. However, sequence analysis of the BV8 CDR3 region showed that the clonotype that dominated the primary response was still detectable, albeit at a lower frequency (44%).

Patient M43 showed an oligoclonal expansion in BV7S1⁺ CD8⁺ T cells (Table 3). The TCRs of these clones showed restricted CDR3 length (9 to 11 amino acids) and joining chain (J) use which was biased toward use of the BJ2S7 gene, 78% compared to 15 to 25% in healthy individuals (29, 30, 38). Extensive analysis of CDR3 sequences associated with BV7S1 in healthy persons has shown a wide range of CDR3 lengths and J β gene usage (4a). This restricted CDR3 pattern is consistent with an antigen-driven CD8⁺ T-cell response (23). In the M43 follow-up sample, BV7S1⁺ CD8⁺ T cells showed a polyclonal response with no clonotype using BJ2S7.

In vaccinee CBL121, the BV17⁺ CD8⁺ T-cell expansion, which consisted of 31.13% CD8⁺ T cells, was predominantly clonal, with 82% of sequences shown to be identical (Table 4).

In this individual the expansion persisted, with the same clonotype continuing to dominate (92%) 32 weeks later.

Expanded T lymphocytes show virus-specific cytotoxic function. To determine whether the expanded T cells were cytotoxic, the patients' PBMC were tested with autologous B cells infected with vaccinia virus expressing MV protein, i.e., fusion protein (F), hemagglutinin (H), or nucleoprotein (NP), at an effector/target ratio of 20:1. Patient M152 had a CD8⁺ T-cell expansion of T cells expressing BV13S1 (9.28% compared to 3.35% \pm 0.96 in Gambian controls) (Table 1) at the time of acute MV infection. PBMC from this patient after overnight culture in the absence of antigen lysed F-expressing vaccinia virus-infected cells. This killing activity was reduced from 30 to 1% after removing the BV13S1⁺ cells from PBMC (Fig. 1).

In another patient, M132, the TCR repertoire, as determined using TCR V β -specific MAbs, was within normal limits. PBMC from this patient lysed H-expressing vaccinia virus-infected target cells. Depletion of BV13S1⁺ T lymphocytes from this patient did not significantly reduce this lytic activity, showing that the antibody and depletion procedure did not nonspecifically reduce cytotoxicity.

Patient M342 had CD8⁺ T-cell expansions of cells expressing BV13S1 (8.06% versus 3.35 \pm 0.96 in Gambian controls) and BV23 (10.89% versus 1.03% \pm 0.50). CTL in this patient showed 12.5 and 20.8% specific cytotoxicity, *ex vivo*, against target cells infected with vaccinia virus expressing F and H, respectively. The activity was decreased to 0 and 2.1% after removing just the expanded BV13S1⁺ and BV23⁺ populations.

Patient M111 had CD8⁺ T-cell expansions of cells expressing BV17 (23% versus 3.8% \pm 1.72), BV13S1 (7.8% versus 3.35% \pm 0.50), BV20 (12% versus 3.01% \pm 2.08), and BV21S3 (7% versus 1.69% \pm 0.97). PBMC from this patient showed killing activity against H-expressing vaccinia virus-infected cells but not against target cells infected with vaccinia virus expressing F and NP. The percent lysis against H of this patient was reduced from 32 to 26% after depletion of BV17⁺ and BV21S3⁺ cells.

DISCUSSION

Distortion of the human T-cell repertoire during acute and chronic infection with HIV, SIV, and EBV has been demonstrated (5, 7, 25, 40). Those reports described expansions in particular V β T-cell subsets during the acute phase of infection. Most of the expansions declined to baseline levels in follow-up samples several weeks later, but in some cases V β T-cell expansions could persist, as detected by PCR, for up to 2 years (40, 41). In order to determine whether skewing of the T-cell repertoire is also characteristic of nonpersistent virus infections, we examined the TCR repertoire of CD8⁺ T cells in patients with acute MV infection. Expansions similar to those detected during primary HIV or EBV infection were found. Recipients of attenuated MV vaccine showed similar results; in two of four vaccinees the T-cell responses were greater than those in naturally infected patients. This may reflect the reduced immunosuppressive effects of the vaccine strain of virus. It is noteworthy that no TCR expansions were found in control children recovering from malaria infection. Although there is good evidence that CD8⁺ T cells respond to liver-stage antigens (27), the response may be very weak compared to that in an acute virus infection.

The TCR β -chain CDR3 region highly variable. It is important in interacting with antigenic peptides presented by HLA molecules (9, 12, 13). Sequence analysis of this highly variable region of the TCR indicates whether the T cells are clonal or oligoclonal, suggesting selection by an antigen, or polyclonal. In patient M42 and vaccinee CBL121, the V β expansions were

TABLE 1. The T-cell repertoire in MV-infected patients and vaccinees

Sample source	HLA A and B type	Sample time ^a	V beta (BV) gene segment usage among CD8 ⁺ T lymphocytes																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
			BV1	BV2	BV3	BV5S2/S3	BV6S7	BV7S1	BV8	BV9	BV12	BV13S1	BV13S2	BV17	BV20	BV21S3	BV23																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
Vaccinee																				CBL108	ND ^c	1st (2)	2.36	3.55	2.08	3.80	0.51	7.48^b	1.54	0.50	0.63	1.37	0.97	21.76	10.53	0.74	15.53					2nd (42)	3.93	3.52	3.85	3.56	0.86	7.98	2.21	0.51	0.64	2.14	1.07	9.60	8.39	1.63	12.34			CBL121	ND	1st (6)	3.39	2.34	0.49	4.18	0.50	1.00	2.70	7.81	0.99	2.16	2.68	31.13	1.08	0.33	1.33					2nd (32)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	30.80	ND	ND	ND			CBL134 (twin 1) ^f	ND	1st (4)	2.71	4.65	1.15	6.13	3.30	3.95	2.66	1.34	1.43	2.50	1.11	3.29	2.71	2.51	0.98			CBL134 (twin 2) ^f	ND	1st (4)	5.90	5.65	0.93	3.85	2.91	1.43	5.72	1.30	2.15	4.32	0.96	4.97	2.57	1.92	0.81			Patient																					M34	ND	A	12.16	4.62	0.27	1.00	0.70	3.20	9.15	1.35	3.80	2.63	0.41	4.49	0.36	1.12	0.86			M35	A2,3; B12,17	A	4.40	4.06	0.66	7.51	1.90	3.35	3.01	4.64	2.50	2.81	2.40	3.53	3.95	3.21	0.82			M36	A19,28; B22,41	A	2.15	3.49	0.00	7.38	0.89	3.70	0.00	0.00	3.61	3.45	0.00	1.49	1.03	0.00	0.78			M37	A9,28; B12,53	A	1.26	0.66	1.98	1.46	0.40	1.85	3.64	1.29	2.12	4.25	0.78	2.67	0.36	6.25	4.07					F (18)	4.36	5.09	1.59	2.63	1.89	1.49	4.29	0.59	1.10	6.04	2.09	4.21	1.03	4.60	1.89			M39	A2,28; B5,35	A	1.57	7.72	6.81	2.89	3.00	2.50	1.17	0.45	0.69	1.80	8.01	1.02	0.70	1.78	21.30					F (24)	5.54	10.37	3.10	2.44	2.72	0.86	3.34	0.48	1.85	2.73	4.58	2.42	0.83	1.20	12.38			M40	A19; B5,35	A	1.39	2.45	10.42	2.61	1.14	3.53	3.67	0.67	1.36	8.08	0.99	3.79	0.37	2.01	0.58			M41	A28; B15	A	3.79	3.23	ND	3.56	0.27	2.91	3.22	0.44	0.24	2.52	1.67	6.07	2.01	2.83	0.89			M42	A19,28; B5,35	A	5.00	3.58	2.41	5.94	0.83	0.59	16.04	0.18	1.28	3.23	0.57	1.98	4.55	2.00	0.32					F (20)	ND	ND	ND	ND	ND	ND	4.60	ND	ND	ND	ND	ND	ND	ND	ND			M43	A1,9; B16	A	8.24	11.45	0.15	2.52	2.20	10.07	4.01	2.27	1.03	2.05	2.21	2.60	1.67	2.05	0.52					F (16)	ND	ND	ND	ND	ND	5.60	ND	ND	ND	ND	ND	ND	ND	ND	ND			M44	A1,10; B8,49	A	6.54	2.58	0.85	0.62	1.20	1.48	3.43	1.69	7.22	2.25	4.09	3.03	0.78	2.01	2.92			M45	A2,3; B7	A	2.17	3.66	1.14	2.35	1.08	2.70	2.15	0.00	0.00	1.32	5.10	3.57	4.17	1.06	4.26					F (23)	5.16	3.65	0.89	3.89	2.11	1.22	4.58	0.31	1.25	3.40	2.65	3.09	1.52	1.05	1.66			M47	A30,32; B35,53	A	2.38	5.62	8.60	6.01	1.57	0.45	13.51	1.58	1.68	2.07	1.45	4.83	1.16	0.82	0.61					F (8)	3.58	3.87	3.95	6.70	1.99	2.31	7.62	ND	ND	ND	ND	ND	1.42	1.99	0.56			M49	A19; B35,22	A	10.36	16.92	8.02	3.54	0.27	1.44	2.73	0.48	6.71	2.76	5.29	4.96	1.35	1.49	0.42					F (15)	7.57	10.19	1.58	4.41	2.16	5.22	3.65	0.99	3.83	2.19	2.82	4.43	1.88	3.33	1.46			M51	A19; B14,35	A	0.98	2.02	1.61	4.71	0.23	0.64	1.19	0.28	12.32	0.69	0.25	0.49	17.71	0.20	11.56			M53	A26,19; B8	A	3.39	5.77	0.63	3.17	0.55	6.11	6.87	1.13	1.56	4.99	1.69	5.52	4.17	1.93	1.40					F (17)	2.39	6.23	1.36	4.76	1.38	3.12	4.73	1.31	1.51	3.57	0.85	7.27	3.29	1.11	1.05			M111	A2,33; B15,50	A	1.92	4.09	1.22	2.08	0.55	0.72	4.34	0.07	1.24	7.80	0.65	23.05	12.34	7.00	5.68			M152	A31,68; B35,53	A	4.21	5.32	6.39	4.39	4.34	3.96	3.40	3.27	0.95	9.28	0.65	3.90	3.03	3.15	3.74			M342	A2,23; B7,53	A	2.73	4.53	5.54	2.75	1.68	3.96	3.02	1.95	1.33	8.06	1.62	4.57	2.64	2.49	10.89			M132		A	7.17	4.67	3.67	3.70	1.18	2.67	5.84	0.44	1.81	0.81	0.96	5.25	2.31	1.24	0.55			10 Gambian controls																					Mean			3.73	4.69	2.20	3.55	2.11	1.77	4.34	0.74	1.51	3.35	1.99	3.80	3.01	1.69	1.03			Standard deviation			1.22	2.13	1.73	2.67	1.78	0.81	2.29	0.47	0.54	0.96	1.44	1.72	2.08	0.97	0.50			Cutoff point ^d			7.40	11.06	7.38	11.57	7.45	4.20	11.22	2.15	3.13	6.22	6.31	8.96	9.24	4.60	2.54			4 Caucasian controls																					Mean			6.46	4.62	3.75	3.88	2.33	2.04	4.51	0.89	1.59	3.54	3.59	3.63	2.42	1.94	1.53			Standard deviation			3.48	1.71	0.90	0.86	1.12	1.26	1.29	0.35	0.63	1.08	1.98	1.70	0.97	0.36	0.75			Cutoff point ^d			16.90	9.75	6.45	6.46	5.69	5.82	8.38	1.94	3.48	6.78	9.53	8.73	5.33	3.02	3.78		
CBL108	ND ^c	1st (2)	2.36	3.55	2.08	3.80	0.51	7.48^b	1.54	0.50	0.63	1.37	0.97	21.76	10.53	0.74	15.53																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
		2nd (42)	3.93	3.52	3.85	3.56	0.86	7.98	2.21	0.51	0.64	2.14	1.07	9.60	8.39	1.63	12.34																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
CBL121	ND	1st (6)	3.39	2.34	0.49	4.18	0.50	1.00	2.70	7.81	0.99	2.16	2.68	31.13	1.08	0.33	1.33																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
		2nd (32)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	30.80	ND	ND	ND																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
CBL134 (twin 1) ^f	ND	1st (4)	2.71	4.65	1.15	6.13	3.30	3.95	2.66	1.34	1.43	2.50	1.11	3.29	2.71	2.51	0.98																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
CBL134 (twin 2) ^f	ND	1st (4)	5.90	5.65	0.93	3.85	2.91	1.43	5.72	1.30	2.15	4.32	0.96	4.97	2.57	1.92	0.81																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
Patient																					M34	ND	A	12.16	4.62	0.27	1.00	0.70	3.20	9.15	1.35	3.80	2.63	0.41	4.49	0.36	1.12	0.86			M35	A2,3; B12,17	A	4.40	4.06	0.66	7.51	1.90	3.35	3.01	4.64	2.50	2.81	2.40	3.53	3.95	3.21	0.82			M36	A19,28; B22,41	A	2.15	3.49	0.00	7.38	0.89	3.70	0.00	0.00	3.61	3.45	0.00	1.49	1.03	0.00	0.78			M37	A9,28; B12,53	A	1.26	0.66	1.98	1.46	0.40	1.85	3.64	1.29	2.12	4.25	0.78	2.67	0.36	6.25	4.07					F (18)	4.36	5.09	1.59	2.63	1.89	1.49	4.29	0.59	1.10	6.04	2.09	4.21	1.03	4.60	1.89			M39	A2,28; B5,35	A	1.57	7.72	6.81	2.89	3.00	2.50	1.17	0.45	0.69	1.80	8.01	1.02	0.70	1.78	21.30					F (24)	5.54	10.37	3.10	2.44	2.72	0.86	3.34	0.48	1.85	2.73	4.58	2.42	0.83	1.20	12.38			M40	A19; B5,35	A	1.39	2.45	10.42	2.61	1.14	3.53	3.67	0.67	1.36	8.08	0.99	3.79	0.37	2.01	0.58			M41	A28; B15	A	3.79	3.23	ND	3.56	0.27	2.91	3.22	0.44	0.24	2.52	1.67	6.07	2.01	2.83	0.89			M42	A19,28; B5,35	A	5.00	3.58	2.41	5.94	0.83	0.59	16.04	0.18	1.28	3.23	0.57	1.98	4.55	2.00	0.32					F (20)	ND	ND	ND	ND	ND	ND	4.60	ND	ND	ND	ND	ND	ND	ND	ND			M43	A1,9; B16	A	8.24	11.45	0.15	2.52	2.20	10.07	4.01	2.27	1.03	2.05	2.21	2.60	1.67	2.05	0.52					F (16)	ND	ND	ND	ND	ND	5.60	ND	ND	ND	ND	ND	ND	ND	ND	ND			M44	A1,10; B8,49	A	6.54	2.58	0.85	0.62	1.20	1.48	3.43	1.69	7.22	2.25	4.09	3.03	0.78	2.01	2.92			M45	A2,3; B7	A	2.17	3.66	1.14	2.35	1.08	2.70	2.15	0.00	0.00	1.32	5.10	3.57	4.17	1.06	4.26					F (23)	5.16	3.65	0.89	3.89	2.11	1.22	4.58	0.31	1.25	3.40	2.65	3.09	1.52	1.05	1.66			M47	A30,32; B35,53	A	2.38	5.62	8.60	6.01	1.57	0.45	13.51	1.58	1.68	2.07	1.45	4.83	1.16	0.82	0.61					F (8)	3.58	3.87	3.95	6.70	1.99	2.31	7.62	ND	ND	ND	ND	ND	1.42	1.99	0.56			M49	A19; B35,22	A	10.36	16.92	8.02	3.54	0.27	1.44	2.73	0.48	6.71	2.76	5.29	4.96	1.35	1.49	0.42					F (15)	7.57	10.19	1.58	4.41	2.16	5.22	3.65	0.99	3.83	2.19	2.82	4.43	1.88	3.33	1.46			M51	A19; B14,35	A	0.98	2.02	1.61	4.71	0.23	0.64	1.19	0.28	12.32	0.69	0.25	0.49	17.71	0.20	11.56			M53	A26,19; B8	A	3.39	5.77	0.63	3.17	0.55	6.11	6.87	1.13	1.56	4.99	1.69	5.52	4.17	1.93	1.40					F (17)	2.39	6.23	1.36	4.76	1.38	3.12	4.73	1.31	1.51	3.57	0.85	7.27	3.29	1.11	1.05			M111	A2,33; B15,50	A	1.92	4.09	1.22	2.08	0.55	0.72	4.34	0.07	1.24	7.80	0.65	23.05	12.34	7.00	5.68			M152	A31,68; B35,53	A	4.21	5.32	6.39	4.39	4.34	3.96	3.40	3.27	0.95	9.28	0.65	3.90	3.03	3.15	3.74			M342	A2,23; B7,53	A	2.73	4.53	5.54	2.75	1.68	3.96	3.02	1.95	1.33	8.06	1.62	4.57	2.64	2.49	10.89			M132		A	7.17	4.67	3.67	3.70	1.18	2.67	5.84	0.44	1.81	0.81	0.96	5.25	2.31	1.24	0.55			10 Gambian controls																					Mean			3.73	4.69	2.20	3.55	2.11	1.77	4.34	0.74	1.51	3.35	1.99	3.80	3.01	1.69	1.03			Standard deviation			1.22	2.13	1.73	2.67	1.78	0.81	2.29	0.47	0.54	0.96	1.44	1.72	2.08	0.97	0.50			Cutoff point ^d			7.40	11.06	7.38	11.57	7.45	4.20	11.22	2.15	3.13	6.22	6.31	8.96	9.24	4.60	2.54			4 Caucasian controls																					Mean			6.46	4.62	3.75	3.88	2.33	2.04	4.51	0.89	1.59	3.54	3.59	3.63	2.42	1.94	1.53			Standard deviation			3.48	1.71	0.90	0.86	1.12	1.26	1.29	0.35	0.63	1.08	1.98	1.70	0.97	0.36	0.75			Cutoff point ^d			16.90	9.75	6.45	6.46	5.69	5.82	8.38	1.94	3.48	6.78	9.53	8.73	5.33	3.02	3.78																																																																																																																																														
M34	ND	A	12.16	4.62	0.27	1.00	0.70	3.20	9.15	1.35	3.80	2.63	0.41	4.49	0.36	1.12	0.86																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M35	A2,3; B12,17	A	4.40	4.06	0.66	7.51	1.90	3.35	3.01	4.64	2.50	2.81	2.40	3.53	3.95	3.21	0.82																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M36	A19,28; B22,41	A	2.15	3.49	0.00	7.38	0.89	3.70	0.00	0.00	3.61	3.45	0.00	1.49	1.03	0.00	0.78																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M37	A9,28; B12,53	A	1.26	0.66	1.98	1.46	0.40	1.85	3.64	1.29	2.12	4.25	0.78	2.67	0.36	6.25	4.07																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
		F (18)	4.36	5.09	1.59	2.63	1.89	1.49	4.29	0.59	1.10	6.04	2.09	4.21	1.03	4.60	1.89																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M39	A2,28; B5,35	A	1.57	7.72	6.81	2.89	3.00	2.50	1.17	0.45	0.69	1.80	8.01	1.02	0.70	1.78	21.30																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
		F (24)	5.54	10.37	3.10	2.44	2.72	0.86	3.34	0.48	1.85	2.73	4.58	2.42	0.83	1.20	12.38																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M40	A19; B5,35	A	1.39	2.45	10.42	2.61	1.14	3.53	3.67	0.67	1.36	8.08	0.99	3.79	0.37	2.01	0.58																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M41	A28; B15	A	3.79	3.23	ND	3.56	0.27	2.91	3.22	0.44	0.24	2.52	1.67	6.07	2.01	2.83	0.89																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M42	A19,28; B5,35	A	5.00	3.58	2.41	5.94	0.83	0.59	16.04	0.18	1.28	3.23	0.57	1.98	4.55	2.00	0.32																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
		F (20)	ND	ND	ND	ND	ND	ND	4.60	ND	ND	ND	ND	ND	ND	ND	ND																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M43	A1,9; B16	A	8.24	11.45	0.15	2.52	2.20	10.07	4.01	2.27	1.03	2.05	2.21	2.60	1.67	2.05	0.52																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
		F (16)	ND	ND	ND	ND	ND	5.60	ND	ND	ND	ND	ND	ND	ND	ND	ND																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M44	A1,10; B8,49	A	6.54	2.58	0.85	0.62	1.20	1.48	3.43	1.69	7.22	2.25	4.09	3.03	0.78	2.01	2.92																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M45	A2,3; B7	A	2.17	3.66	1.14	2.35	1.08	2.70	2.15	0.00	0.00	1.32	5.10	3.57	4.17	1.06	4.26																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
		F (23)	5.16	3.65	0.89	3.89	2.11	1.22	4.58	0.31	1.25	3.40	2.65	3.09	1.52	1.05	1.66																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M47	A30,32; B35,53	A	2.38	5.62	8.60	6.01	1.57	0.45	13.51	1.58	1.68	2.07	1.45	4.83	1.16	0.82	0.61																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
		F (8)	3.58	3.87	3.95	6.70	1.99	2.31	7.62	ND	ND	ND	ND	ND	1.42	1.99	0.56																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M49	A19; B35,22	A	10.36	16.92	8.02	3.54	0.27	1.44	2.73	0.48	6.71	2.76	5.29	4.96	1.35	1.49	0.42																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
		F (15)	7.57	10.19	1.58	4.41	2.16	5.22	3.65	0.99	3.83	2.19	2.82	4.43	1.88	3.33	1.46																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M51	A19; B14,35	A	0.98	2.02	1.61	4.71	0.23	0.64	1.19	0.28	12.32	0.69	0.25	0.49	17.71	0.20	11.56																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M53	A26,19; B8	A	3.39	5.77	0.63	3.17	0.55	6.11	6.87	1.13	1.56	4.99	1.69	5.52	4.17	1.93	1.40																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
		F (17)	2.39	6.23	1.36	4.76	1.38	3.12	4.73	1.31	1.51	3.57	0.85	7.27	3.29	1.11	1.05																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M111	A2,33; B15,50	A	1.92	4.09	1.22	2.08	0.55	0.72	4.34	0.07	1.24	7.80	0.65	23.05	12.34	7.00	5.68																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M152	A31,68; B35,53	A	4.21	5.32	6.39	4.39	4.34	3.96	3.40	3.27	0.95	9.28	0.65	3.90	3.03	3.15	3.74																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M342	A2,23; B7,53	A	2.73	4.53	5.54	2.75	1.68	3.96	3.02	1.95	1.33	8.06	1.62	4.57	2.64	2.49	10.89																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
M132		A	7.17	4.67	3.67	3.70	1.18	2.67	5.84	0.44	1.81	0.81	0.96	5.25	2.31	1.24	0.55																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
10 Gambian controls																					Mean			3.73	4.69	2.20	3.55	2.11	1.77	4.34	0.74	1.51	3.35	1.99	3.80	3.01	1.69	1.03			Standard deviation			1.22	2.13	1.73	2.67	1.78	0.81	2.29	0.47	0.54	0.96	1.44	1.72	2.08	0.97	0.50			Cutoff point ^d			7.40	11.06	7.38	11.57	7.45	4.20	11.22	2.15	3.13	6.22	6.31	8.96	9.24	4.60	2.54			4 Caucasian controls																					Mean			6.46	4.62	3.75	3.88	2.33	2.04	4.51	0.89	1.59	3.54	3.59	3.63	2.42	1.94	1.53			Standard deviation			3.48	1.71	0.90	0.86	1.12	1.26	1.29	0.35	0.63	1.08	1.98	1.70	0.97	0.36	0.75			Cutoff point ^d			16.90	9.75	6.45	6.46	5.69	5.82	8.38	1.94	3.48	6.78	9.53	8.73	5.33	3.02	3.78																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															
Mean			3.73	4.69	2.20	3.55	2.11	1.77	4.34	0.74	1.51	3.35	1.99	3.80	3.01	1.69	1.03																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
Standard deviation			1.22	2.13	1.73	2.67	1.78	0.81	2.29	0.47	0.54	0.96	1.44	1.72	2.08	0.97	0.50																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
Cutoff point ^d			7.40	11.06	7.38	11.57	7.45	4.20	11.22	2.15	3.13	6.22	6.31	8.96	9.24	4.60	2.54																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
4 Caucasian controls																					Mean			6.46	4.62	3.75	3.88	2.33	2.04	4.51	0.89	1.59	3.54	3.59	3.63	2.42	1.94	1.53			Standard deviation			3.48	1.71	0.90	0.86	1.12	1.26	1.29	0.35	0.63	1.08	1.98	1.70	0.97	0.36	0.75			Cutoff point ^d			16.90	9.75	6.45	6.46	5.69	5.82	8.38	1.94	3.48	6.78	9.53	8.73	5.33	3.02	3.78																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
Mean			6.46	4.62	3.75	3.88	2.33	2.04	4.51	0.89	1.59	3.54	3.59	3.63	2.42	1.94	1.53																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
Standard deviation			3.48	1.71	0.90	0.86	1.12	1.26	1.29	0.35	0.63	1.08	1.98	1.70	0.97	0.36	0.75																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
Cutoff point ^d			16.90	9.75	6.45	6.46	5.69	5.82	8.38	1.94	3.48	6.78	9.53	8.73	5.33	3.02	3.78																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													

^a 1st, 2nd, A, and F represent first, second, acute, and follow-up samples, respectively. The number in parentheses indicates weeks after vaccination or infection.

^b Percentage of a particular TCR Vβ shown in bold was considered to be expanded.

^c These children are twins.

^d The cutoff point for a particular TCR Vβ family was calculated as the mean plus 3 standard deviations among controls (10 Gambian children recovering from malaria infection or 4 healthy Caucasian controls).

^e ND, not done.

dominated by a single clone, whereas in patient M43 the expanded BV7S1⁺ CD8⁺ T cells showed oligoclonality, with selection for CDR3 length and heavy bias toward the use of joining chain BJ2S7. This joining chain is one of two Jβ families containing a glutamate-glutamine motif (highlighted in Table 3) (35) which has been shown to be involved in antigen recognition by the TCR (19). The clonal and oligoclonal nature of the expansions found during primary MV infection strongly suggests that they are driven by antigen specificity.

Following recovery from MV infection, the T-cell repertoire, as determined using TCR Vβ-specific MAbs, usually returned to normal. However, a few expansions sometimes remained.

Half the vaccinees showed results similar to those infected naturally, with some persistent, oligoclonal T-cell expansions. Chronic clonal expansion in a persistent infection probably results from continuous activation by antigens; however, in MV infection the virus disappears from the blood and tissue within 1 month (17), yet the expansions can persist much longer. Why these clones persist and escape from activation-induced cell death is not known. Not all clones persist, however, for example, the BV7S1 population in M43 was lost.

Until now it has been difficult to show that the expanded CD8⁺ T cells found in acute virus infections can mediate antigen-specific cytotoxicity (5, 25). Recently, staining of ex-

TABLE 2. Analysis of BV8TCR sequences in acute and follow-up samples in patient M42

Sample time	End sequence of BV8	CDR3	BJ	Frequency
Acute infection ^a	CAS	SPGGIGAF	FG 1S1	7/11
	CAS	SLGGREQY	FG 2S7	1/11
	CAS	SSYSEAF	FG 1S1	1/11
	CAS	SPDBRGEPTDTQY	FG 2S3	1/11
	CAS	SLAGPNEKLF	FG 1S4	1/11
Follow-up ^b	CAS	SPGGIGAF	FG 1S1	4/9
	CAS	SFWGLGETQY	FG 2S5	1/9
	CAS	TPPRDRPISPOH	FG 1S5	1/9
	CAS	SSWEPGIVTEAF	FG 1S1	1/9
	CAS	SSTAENSPLH	FG 1S6	1/9
	CAS	SPTLGRGDSNAGELF	FG 2S2	1/9

^a At this time, 16.0% of CD8⁺ T cells were expressing BV8.

^b At the 20-week follow-up, 4.6% of CD8⁺ T cells were expressing BV8.

panded T cells with tetrameric HLA-peptide complexes has confirmed that they are antigen specific (6, 11, 41), and when sorted by flow cytometry, these T cells secrete gamma interferon in response to antigen challenge (10). Pantaleo et al. (25) showed cytotoxic capacity in sorted CD8⁺ T cells expressing BV17, an expanded cell population in an HIV patient. However, the sorted cells had to be stimulated in culture with an anti-CD3 MAb for 10 days, and a surprisingly high effector-to-target (E:T) cell ratio was needed to demonstrate significant killing. Here, we tested the virus-specific cytotoxic activity of fresh unstimulated PBMC before and after depleting the expanded population. When the expanded T cells were removed from PBMC of patients with acute MV infection, cytotoxic activity against target cells infected with MV-expressing vaccinia virus was completely lost. The antibody only removed the T cells carrying that receptor, and the BV13S1 antibody only impaired the lytic response when there was a BV13S1 expansion, excluding nonspecific effects. This study therefore pro-

TABLE 3. Analysis of BV7S1 TCR sequences in acute and follow-up samples in patient M43

Sample time	End sequence of BV7S1	CDR3 ^c	BJ	Frequency	
Acute infection ^a	CAS	SQKMVPTPYT	FG 1S2	4/18	
	CAS	SQGDRVNEQF	FG 2S1	3/18	
	CAS	SQVGGQETQY	FG 2S5	2/18	
	CAS	SQERRQKQY	FG 2S7	1/18	
	CAS	SQEGWYEQY	FG 2S7	1/18	
	CAS	SPQRDRAYEQY	FG 2S7	1/18	
	CAS	SQGQANYEQY	FG 2S7	1/18	
	CAS	SPGTGRPEQY	FG 2S7	1/18	
	CAS	SPGDRVYEQY	FG 2S7	1/18	
	CAS	RKDRGTYEQY	FG 2S7	1/18	
	CAS	SQVWTGDQPOH	FG 1S5	1/18	
	CAS	SQEGAGGDSTNEKLF	FG 1S4	1/18	
	Follow-up ^b	CAS	SQGQADISRGYT	FG 1S2	2/9
		CAS	SQKMVPTPYT	FG 1S2	1/9
CAS		SPTQGGGEKLF	FG 1S4	1/9	
CAS		SQTTTSTYSPLH	FG 1S6	1/9	
CAS		SQDMVGLAVDEQF	FG 2S1	1/9	
CAS		SQWTPSEAF	FG 1S1	1/9	
CAS		SQVQDTEAF	FG 1S1	1/9	
CAS		SNNWGVTEAF	FG 1S1	1/9	

^a At this time, 10.1% of CD8⁺ T cells were expressing BV7S1.

^b At the 16-week follow-up, 5.6% of CD8⁺ T cells were expressing BV7S1.

^c Glutamate-glutamine motif is highlighted.

TABLE 4. Analysis of BV17 TCR sequences in acute and follow-up samples in vaccinee CBL121

Sample	End sequence of BV17	CDR3	BJ	Frequency
First ^a	CAS	SHDGYTEAF	FG 1S1	9/11
	CAS	GPAEAMNTEAF	FG 1S1	1/11
	CAS	SPDNQPQH	FG 1S5	1/11
Second ^b	CAS	SHDGYTEAF	FG 1S1	11/12
	CAS	NPGYGYT	FG 1S2	1/12

^a At 6 weeks after vaccination, 31.1% of CD8⁺ T cells were expressing BV17.

^b At the 32-week follow-up, 30.1% of CD8⁺ T cells were expressing BV17.

vides direct evidence that the expanded CD8⁺ T cells are specific for MV antigens and function as cytotoxic effector cells.

The magnitude of the clonal expansions which develop in response to acute virus infection is very variable. Massive clonal expansions could lead to exhaustion and anergy (22, 26). Gallimore et al. (11) showed reduced function in some expanded CD8⁺ T-cell populations in mice after acute infection with an aggressive variant of lymphocytic choriomeningitis virus. In patients M152 and M342, expansions comprised 8 to 11% CD8⁺ T lymphocytes. Cytotoxicity was abolished after depleting these particular populations. In contrast, in patient M111, no significant cytotoxicity was found in the expanded T cells. This patient had four expansions, the largest against BV17, consisting of 23% of the CD8⁺ T cells. PBMC from this patient killed target cells expressing H but not those expressing F and NP. The killing activity was only slightly decreased after depleting cells expressing BV17 and BV21S3 which together comprised 30% of CD8⁺ T cells. Thus, the specificity for H probably resided in the smaller expansions. The depleted T-cell clones might react to other MV proteins which were not tested, although Jaye et al. (18) have shown that F, H, and NP are normally the major targets for CTL. Alternatively, the large, expanded BV17 and BV21S3 T-cell population in this patient might comprise cells at a late stage of differentiation with poor cytotoxic function (8). Thus, although the expanded CD8⁺ T cells can be shown in some cases to have very specific cytotoxic function, it is possible that further expansion could lead to a decrease in function. This issue needs further examination. A third possibility is that these cells are bystander

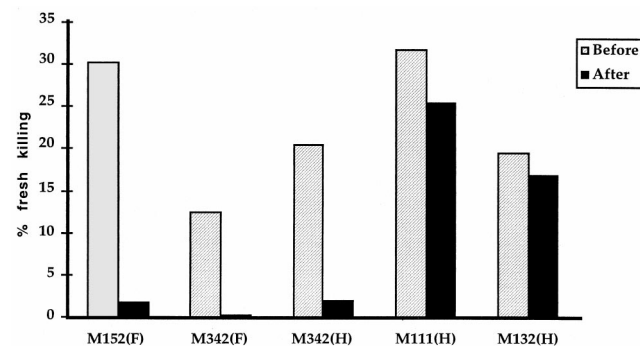


FIG. 1. Cytotoxic activity from patients with acute MV infection was tested before and after depleting V β expansions from PBMC. The percent specific lysis of target cells expressing vaccinia virus recombinants expressing fusion protein (F) and hemagglutinin (H) was shown. In patients M152 and M342, all the expanded T cells were removed. In patient M111, only 2 of 4 V β expansions were depleted. Patient M132, who had no particular TCR expansion, was used as negative control. PBMC of these patients were also tested for cytotoxic activity against β -galactosidase-expressing vaccinia virus, on a different occasion, as a control. All samples had less than 5% killing against this recombinant.

expansions in response to cytokines; although bystander effects have been described previously (34), such expansions are not oligoclonal and their magnitude has been questioned (4, 24).

In conclusion, we have demonstrated large expansions of virus-specific CD8⁺ T-cell clones that have cytolytic activity in acute MV infection. Similar expansions were found after vaccination with live, attenuated MV. In many cases, the expansions persisted for several months. Such expansions comprise a few clones and are remarkable for their magnitude. It is likely that they contribute to the control and clearance of MV infection.

ACKNOWLEDGMENTS

We thank Siriraj Hospital Faculty of Medicine, Mahidol University Thailand, a Training Research Fellowship from Rockefeller Foundation, and the Medical Research Council for support of personnel and expenses.

We thank P. Marrack (Howard Hughes Medical Institute Research Laboratories) for BV13S1 and BV13S2 antibodies, F. Wild (Institute Pasteur de Lyon) for the vaccinia virus recombinants, and L. Tan for unpublished data. We also acknowledge T. Corrah, A. Sadiq, M. Ngong, and C. Vimteh for excellent field work, and finally, we are very grateful to all the subjects and controls.

REFERENCES

- Axton, J. H. 1979. Measles and the state of nutrition. *S. Afr. Med. J.* **55**:125-126.
- Beckford, A. P., R. O. Kaschula, and C. Stephen. 1985. Factors associated with fatal cases of measles. A retrospective autopsy study. *S. Afr. Med. J.* **68**:858-863.
- Burnet, F. M. 1968. Measles as an index of immunological function. *Lancet* **ii**:610-613.
- Butz, E. A., and M. J. Bevan. 1998. Massive expansion of antigen-specific CD8⁺ T cells during an acute virus infection. *Immunity* **8**:167-175.
- Callan, M., and L. Tan. Unpublished data.
- Callan, M. F., N. Steven, P. Krausa, J. D. Wilson, P. A. Moss, G. M. Gillespie, J. I. Bell, A. B. Rickinson, and A. J. McMichael. 1996. Large clonal expansions of CD8⁺ T cells in acute infectious mononucleosis. *Nat. Med.* **2**:906-911.
- Callan, M. F., L. Tan, N. Anells, G. S. Ogg, J. D. Wilson, C. A. O'Callaghan, N. Steven, A. J. McMichael, and A. B. Rickinson. 1998. Direct visualization of antigen-specific CD8(+) T cells during the primary immune response to Epstein-Barr virus in vivo. *J. Exp. Med.* **187**:1395-1402.
- Chen, Z. W., Z. C. Kou, C. Lekutis, L. Shen, D. Zhou, M. Halloran, J. Li, J. Sodroski, D. Lee-Parritz, and N. L. Letvin. 1995. T cell receptor v β repertoire in an acute infection of rhesus monkeys with simian immunodeficiency viruses and a chimeric simian-human immunodeficiency virus. *J. Exp. Med.* **182**:21-31.
- d'Angeac, A. D., S. Monier, D. Pilling, A. Travaglio Encinoza, T. Reme, and M. Salmon. 1994. CD57⁺ T lymphocytes are derived from CD57⁻ precursors by differentiation occurring in late immune responses. *Eur. J. Immunol.* **24**:1503-1511.
- Ding, Y. H., K. J. Smith, D. N. Garbozi, U. Utz, W. E. Biddison, and D. C. Wiley. 1998. Two human T cell receptors bind in a similar diagonal mode to the HLA-A2/Tax peptide complex using different TCR amino acids. *Immunity* **8**:403-411.
- Dunbar, P. R., G. S. Ogg, J. Chen, N. Rust, P. van der Bruggen, and V. Cerundolo. 1998. Direct isolation, phenotyping and cloning of low-frequency antigen-specific cytotoxic T lymphocytes from peripheral blood. *Curr. Biol.* **8**:413-416.
- Gallimore, A., A. Glithero, A. Godkin, A. C. Tissot, A. Pluckthun, T. Elliott, H. Hengartner, and R. Zinkernagel. 1998. Induction and exhaustion of lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I-peptide complexes. *J. Exp. Med.* **187**:1383-1393.
- Garbozi, D. N., P. Ghosh, U. Utz, Q. R. Fan, W. E. Biddison, and D. C. Wiley. 1996. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. *Nature* **384**:134-141.
- Garcia, K. C., M. Degano, R. L. Stanfield, A. Brunmark, M. R. Jackson, P. A. Peterson, L. Teyton, and I. A. Wilson. 1996. An alpha T cell receptor structure at 2.5 Å and its orientation in the TCR-MHC complex. *Science* **274**:209-219.
- Gellin, B. G., and S. L. Katz. 1994. Putting a stop to a serial killer: measles. *J. Infect. Dis.* **170**:S1-S2.
- Gellin, B. G., and S. L. Katz. 1994. Measles: state of the art and future directions. *J. Infect. Dis.* **170**:S3-S14.
- Greenstein, J. I., and H. F. McFarland. 1983. Response of human lymphocytes to measles virus after natural infection. *Infect. Immun.* **40**:198-204.
- Griffin, D. E., B. J. Ward, and L. M. Esolen. 1994. Pathogenesis of measles virus infection: an hypothesis for altered immune responses. *J. Infect. Dis.* **170**:S24-S31.
- Jaye, A., A. F. Magnusen, and H. C. Whittle. 1998. Human leukocyte antigen class I- and class II-restricted cytotoxic T lymphocyte responses to measles antigens in immune adults. *J. Infect. Dis.* **177**:1282-1289.
- Jorgensen, J. L., P. A. Reay, E. W. Ehrlich, and M. M. Davis. 1992. Molecular components of T-cell recognition. *Annu. Rev. Immunol.* **10**:835-873.
- Miller, D. 1963. The frequency of complications of measles. *Br. Med. J.* **2**:75-78.
- Morley, D. 1973. Severe measles in Africa, p. 207-230. Butterworths, London, United Kingdom.
- Moskophidis, D., F. Lechner, H. Pircher, and R. M. Zinkernagel. 1993. Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. *Nature* **362**:758-761.
- Moss, P. A., R. J. Moots, W. M. Rosenberg, S. J. Rowland-Jones, H. C. Bodmer, A. J. McMichael, and J. I. Bell. 1991. Extensive conservation of alpha and beta chains of the human T-cell antigen receptor recognizing HLA-A2 and influenza A matrix peptide. *Proc. Natl. Acad. Sci. USA* **88**:8987-8990.
- Murali-Krishna, K., J. D. Altman, M. Suresh, D. J. D. Sourdive, A. J. Zajac, J. D. Miller, J. Slansky, and R. Ahmed. 1998. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. *Immunity* **8**:177-187.
- Pantaleo, G., J. F. Demarest, H. Soudeyns, C. Graziosi, F. Denis, J. W. Adelsberger, P. Borrow, M. S. Saag, G. M. Shaw, R. P. Sekaly, and A. S. Fauci. 1994. Major expansion of CD8⁺ T cells with a predominant V beta usage during the primary immune response to HIV. *Nature* **370**:463-467. Comments.
- Pantaleo, G., H. Soudeyns, J. F. Demarest, M. Vaccarezza, C. Graziosi, S. Paolucci, M. Daucher, O. J. Cohen, F. Denis, W. E. Biddison, R. P. Sekaly, and A. S. Fauci. 1997. Evidence for rapid disappearance of initially expanded HIV-specific CD8⁺ T cell clones during primary HIV infection. *Proc. Natl. Acad. Sci. USA* **94**:9848-9853.
- Plebanski, M., M. Aidoo, H. C. Whittle, and A. V. Hill. 1997. Precursor frequency analysis of cytotoxic T lymphocytes to pre-erythrocytic antigens of *Plasmodium falciparum* in West Africa. *J. Immunol.* **158**:2849-2855.
- Posnett, D. N., R. Sinha, S. Kabak, and C. Russo. 1994. Clonal populations of T cells in normal elderly humans: the T cell equivalent to "benign monoclonal gammopathy." *J. Exp. Med.* **179**:609-618.
- Quiros Roldan, E., A. Sottini, A. Bettinardi, A. Albertini, L. Imberti, and D. Primi. 1995. Different TCRBV genes generate biased patterns of V-D-J diversity in human T cells. *Immunogenetics* **41**:91-100.
- Rosenberg, W. M., P. A. Moss, and J. I. Bell. 1992. Variation in human T cell receptor V beta and J beta repertoire: analysis using anchor polymerase chain reaction. *Eur. J. Immunol.* **22**:541-549.
- Samb, B., P. Aaby, H. Whittle, A. M. Seck, and F. Simondon. 1997. Decline in measles case fatality ratio after the introduction of measles immunization in rural Senegal. *Am. J. Epidemiol.* **145**:51-57.
- Samsi, T. K., T. Ruspanji, I. Susanto, and K. Gunawan. 1992. Risk factors for severe measles. Southeast Asian J. Trop. Med. Public Health **23**:497-503.
- Tamashiro, V. G., H. H. Perez, and D. E. Griffin. 1987. Prospective study of the magnitude and duration of changes in tuberculin reactivity during uncomplicated and complicated measles. *Pediatr. Infect. Dis. J.* **6**:451-454.
- Tough, F. D., P. Borrow, and J. Sprent. 1996. Induction of bystander T cell proliferation by virus and type I interferon in vivo. *Science* **272**:1947-1950.
- Toyonaga, B., Y. Yoshikai, V. Vadasz, B. Chin, and T. W. Mak. 1985. Organization and sequences of the diversity, joining, and constant region genes of the human T-cell receptor beta chain. *Proc. Natl. Acad. Sci. USA* **82**:8624-8628.
- van Binnendijk, R. S., M. C. Poelen, K. C. Kuijpers, A. D. Osterhaus, and F. G. Uytendaele. 1990. The predominance of CD8⁺ class I MHC-restricted cytotoxic T lymphocytes (CTL) in recovery from measles. Clonal analyses of human CD8⁺ class I MHC-restricted CTL. *J. Immunol.* **144**:2394-2399.
- von Pirquet, C. 1908. Das Verhalten der kutanen Tuberkulin-Reaktion wahr-ender Masern. *Deutsch Med. Wochenschr.* **343**:1297-1300.
- Walser Kuntz, D. R., C. M. Weyand, A. J. Weaver, W. M. O'Fallon, and J. J. Goronzy. 1995. Mechanisms underlying the formation of the T cell receptor repertoire in rheumatoid arthritis. *Immunity* **2**:597-605.
- Ward, B. J., R. T. Johnson, A. Vaisberg, E. Jauregui, and D. E. Griffin. 1990. Spontaneous proliferation of peripheral mononuclear cells in natural measles virus infection: identification of dividing cells and correlation with mitogen responsiveness. *Clin. Immunol. Immunopathol.* **55**:315-326.
- Wilson, J. D. K., M. Cranage, N. Cook, S. Leech, A. J. McMichael, and M. F. C. Callan. 1998. Evidence for the persistence of monoclonal expansions of CD8⁺ T cells following primary simian immunodeficiency virus infection. *Eur. J. Immunol.* **28**:1172-1180.
- Wilson, J. D. K., G. Ogg, R. L. Allen, P. J. Goulder, T. Kelleher, A. Sewell, C. A. E. O'Callaghan, S. L. Rowland-Jones, and A. J. McMichael. 1998. Oligoclonal expansions of CD8⁺ T cells in chronic HIV infection are antigen specific. *J. Exp. Med.* **188**:785-790.
- Yanagi, Y., B. A. Cubitt, and M. B. Oldstone. 1992. Measles virus inhibits mitogen-induced T cell proliferation but does not directly perturb the T cell activation process inside the cell. *Virology* **187**:280-289.