0022-1767/89/1428-2841$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 142, 2841–2846, No. 8, April 15, 1989
Printed in U.S.A.

MEASLES VIRUS-SPECIFIC MURINE T CELL CLONES:

Characterization of Fine Specificity and Function[1]

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Measles virus (MV)-specific murine helper T cell clones (Thy-1.2$^+$, CD4$^+$, CD8$^-$) were generated from mice immunized with MV-infected mouse brain homogenate by limiting dilution and in vitro stimulation of spleen cells with UV-inactivated MV Ag. The protein specificity of 7 out of 37 stable T cell clones, which displayed MHC-restricted MV Ag recognition, could be assessed by using purified MV proteins. Two fusion (F) protein-specific, two hemagglutinin-specific, and three nucleoprotein- or matrix protein-specific clones were shown to be established. The F protein-specific T cell clones together with a panel of previously generated F protein-specific T cell clones were characterized for their fine specificity by using β-galactosidase fusion products, which contained different parts of the F protein. It was shown that at least two epitopes on the major part of the F protein (amino acid 2-513) can be recognized by mouse T cells. Functional characterization of three T cell clones showed that they were able to assist MV-specific B cells and bystander B cells for antibody production. Furthermore, they were shown to produce the lymphokines IL-2 and IFN-γ. It was also shown that these T cell clones induced a MV-specific delayed type hypersensitivity response. These observations suggest that all of the T cell clones characterized belong to the T_H1 helper subset.

Measles is a highly contagious disease of major biological importance to humans. Both humoral and cell-mediated immunity are involved in the protection against and recovery from measles virus (MV)[3] infection. Because we are interested in the development of an inactivated measles vaccine, it is of major importance to understand the role of the individual viral proteins in the induction of protective immunity. Both of the glycoproteins of MV, the hemagglutinin (H) protein and the fusion (F) protein, which are expressed on the viral and cell membranes, have been shown to be responsible for the induction of virus-neutralizing antibodies (1–3). In addition, the F protein elicits hemolysis-inhibiting antibodies, which are also thought to play an important role in protection (4). Although there is evidence that MV-specific, cell-mediated immunity plays a role in protection against disease, relatively little is known about the contribution of the individual viral proteins involved. In rats it has been demonstrated that the H and F proteins together are able to induce an in vitro primary and anamnestic cellular response, depending on the Ag presentation form of the proteins used (5). We have shown that the F protein induces a MV-specific delayed type hypersensitivity (DTH) response in mice and that MV-F-specific T cells could be cloned from these animals (6). Human T cell clones directed against different MV proteins have been established and functionally characterized in several in vitro assays (7–9).[4] However, the role of such T cells in in vivo protection cannot be evaluated in humans. Therefore, we have established a MV infection model in mice (6) and we have started to investigate the role of B and T cell-mediated immunity in protection against MV infection in this model. In the present paper we describe the establishment of MV-specific murine T cell clones against different MV proteins. These clones were, together with a previously generated panel of F-specific T cell clones, characterized for their specificity in in vitro assays, by using purified MV proteins and F protein β-galactosidase fusion products. They have also been functionally tested in in vitro and in vivo systems.

Received for publication July 12, 1988.
Accepted for publication January 24, 1989.
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[1] This investigation was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).
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[3] Abbreviations used in this paper: MV, measles virus; F, fusion protein; H, hemagglutinin protein; HI, hemagglutination inhibiting; IMDM-S, Iscove's modified Dulbecco's medium with supplements; iscom, immune stimulating complex; M, matrix protein; MBH, mouse brain homogenate; N, nucleoprotein; RBH, rat brain homogenate; RV, rabies virus.

MATERIALS AND METHODS

Mice. BALB/c mice, 6 to 12 weeks old, were bred at the animal facilities in the National Institute of Public Health and Environmental Protection (Bilthoven, The Netherlands) and BALB/b, B10.D2, and C57BL/6J mice, 6 to 8 weeks old, were purchased from Harlan/Olac (Bicester, Oxon, UK).

Viruses. MV-infected mouse brain homogenate (CAM-MBH) containing 5×10^5 $TCID_{50}$/ml was prepared 5 days after intracerebral inoculation of newborn (1- to 2-day-old) BALB/c mice with 20 μl of a

[4] **Van Binnendijk, R. S., M. C. M. Poelen, P. de Vries, H. O. Voorma, A. D. M. E. Osterhaus, and F. G. C. M. Uytdehaag.** 1989. Measles virus-specific human T cell clones: characterization of specificity and function of CD_4^+ helper/cytotoxic and CD_8^+ cytotoxic T cell clones. *J. Immunol. 142:2847.*

2.5% (w/v) brain suspension of rat brain-adapted MV (CAM-RBH), which was kindly provided by Dr. V. Ter Meulen (Institut für Virologie und Immunobiologie, Würzburg, FRG) (10). MV (Edmonston B strain) was propagated in Vero cells, purified by gradient centrifugation and UV-irradiated with a dose sufficient to inactivate the virus, but preserving other biological properties such as hemagglutination and hemolyzing activities, as previously described (6). Rabies virus (RV, strain Wistar PM/W 1-38-1503-3m) was propagated in dog kidney cells, concentrated and inactivated with β-propiolactone as described (11).

Ag preparations. UV-inactivated MV, propagated in Vero cells, was routinely used for the generation, maintenance, and characterization of T cells. The purification of the F protein and of the H protein by immunoaffinity chromatography, the protein composition of the nucleo and matrix (N/M) protein preparation, and the Vero cell lysate used in the T cell proliferative assay were previously described (6, 12). β-Galactosidase fusion proteins, which consisted of both the β-galactosidase protein and deletion products of the F protein, were expressed from pEX3 constructs containing sequences encoding parts of the F protein (13). Briefly, the 5′ leader sequence of a complete copy of the gene encoding for the F protein, isolated from a constructed cDNA bank, was removed up to the first ATG by using *Bal*31 exonuclease before ligation in the *Bam*HI site of pEX3. pEX clone 117 contained the sequence encoding for amino acid 2-513 of the F protein. The 3′ deletions of this sequence were generated by partial digestion of the pEX clone 117 with either restriction enzyme *Taq*1 or *Rsa*1 and complete digestion with restriction enzyme *Xba*1, followed by re-ligation after fractionation on 0.8% low melting point agarose gel and filling in the sticky ends. A set of different deletions was selected on the basis of restriction fragment analysis and SDS-PAGE and Western blot analysis of the expressed β-galactosidase fusion proteins. Ag used in the T cell proliferative assay were isolated from *Escherichia coli* AB1157 carrying the plasmid pEX3 or derivates. The cells induced to produce the various β-galactosidase F proteins were lysed with lysozyme (1 g/liter) for 40 min at 0°C and subsequently for 5 min with 0.11% Triton X-100 in 25 mM Tris-HCl, pH 8.0, 10 mM EDTA, and 50 mM glucose at 0°C. After centrifugation (20 min, 10,000 rpm), the pellet was resuspended in 0.1% Triton X-100 in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.5). Triton X-100 was removed from the β-galactosidase fusion proteins by centrifugation of the proteins through a 20% sucrose cushion in TE buffer (10 min, 10,000 rpm). Pellets were resuspended in PBS and ultrasonicated three times for 15 sec each, at full power. About 90% of the total protein in the preparations consisted of F protein. The final amount of protein used for T cell stimulation was 0.3 μg/well.

Generation of T cell clones. BALB/c mice, 8 weeks of age, were immunized once i.p. with about 10^5 $TCID_{50}$ of CAM-MBH, 5, 6, or 7 days before the spleens were isolated. Establishment and maintenance of T cell clones were carried out essentially as described for the generation of T cell clones by F immune stimulating complex (iscom) (6). Briefly, 1.5×10^6 spleen cells isolated by density sedimentation were cultured in Iscove's modified Dulbecco's medium, supplemented with glutamine, penicillin, streptomycin, and 2-mercaptoethanol (IMDM-S), 10% FCS, and 5% IL-2 containing supernatant from rat splenocytes, and in the presence of 0.5 or 20μg of UV-inactivated MV. After 5 days of cultivation, lymphoblastoid cells were isolated by gradient centrifugation on Percoll (14). T cell clones were obtained from blastoid cells, by culturing the cells at limiting dilution in IMDM-S supplemented with 10% FCS and 5% IL-2 containing supernatant and in the presence of 0.5 μg of UV-inactivated MV and 3×10^5 irradiated syngeneic spleen cells per well. T cells were maintained in culture by weekly restimulation with fresh irradiated spleen cells and MV Ag. T cell clones were considered to be stable if they could be maintained for more than 8 weeks in tissue culture.

The F-specific T cell clones 0.5 E10, 20 F1, and 20 F3 have previously been described, and the F-specific T cell clones MV2 H10 and F3 A1 were also established from F iscom-immunized mice essentially as previously described (6).

T cell proliferative assay. Viable T cells were separated from non-viable cells 7 to 9 days after Ag stimulation by sedimentation on a density gradient and were resuspended in IMDM-S supplemented with 10% FCS. T cells (1×10^4) were cultured in 96-well, round-bottomed microtiter plates in the presence of 3×10^5 irradiated spleen cells, which had been incubated before for 2 h with the appropriate Ag. Of UV-inactivated MV, 0.6 μg was added to each well, and of the purified Ag, control Ag, or the β-galactosidase F proteins, 0.3 μg was added to each well. After 48 h incubation at 37°C in a humidified atmosphere of 5% CO_2 in air, cultures were pulsed with 0.5 μCi of [^{3}H]thymidine and harvested onto glass fiber filters 18 h later. [^{3}H]Thymidine incorporation was measured by liquid scintillation spectroscopy.

Phenotypic analysis of T cells. Viable T cells were incubated with rat mAb directed against Thy-1.2, CD4, or CD8 cell markers, washed, and subsequently incubated with a FITC-conjugated goat anti-rat κ-chain antibody according to the procedure recommended by the manufacturer (Becton-Dickinson Immunocytometry Systems). The surface phenotype was analyzed by FMF and the Consort-30 program (Becton-Dickinson).

Assay for Th activity in vitro. Spleens from BALB/c mice immunized twice s.c. at an interval of 4 weeks with 30 μg of UV-inactivated MV, in CFA or IFA, respectively, were isolated and homogenized 10 weeks after the last immunization. Splenocytes were depleted of T cells by treatment with the rat mAb anti-Thy-1.2 and Low Tox rabbit complement (Cedarlane Laboratories, Hornby, Canada) according to methods recommended by the manufacturers. These cells were co-cultured at 5×10^5 cells/well with 10^2, 10^3, or no T cells and 7 ng of MV or no Ag in flat-bottomed plates. After 7 days the culture supernatants were harvested and assayed for the presence of anti-viral IgG antibodies in MV-ELISA and for MV-hemagglutination inhibiting (MV-HI) antibodies as previously described (6). In a second assay the MV-primed B cells were mixed (1/1) with T cell-depleted spleen cells from BALB/c mice, immunized twice at an interval of 4 weeks with 150 μg of RV, respectively. This mixed spleen cell population was co-cultured at 5×10^5 cells/well with 10^2 T cells, or without T cells and in the presence of 7 ng of MV, 1 μg of RV, or both MV and RV as stimulating Ag. Supernatants were assayed in MV-ELISA and in RV-ELISA, as previously described (15).

Lymphokine assays. T cells (10^5 cells) were co-cultured with 3×10^5 irradiated spleen cells, which had been incubated before for 2 h with UV-inactivated MV (0.6 μg) or with control Ag. Supernatants were collected 18 h after cultivation. IL-2 was assayed as previously described (16) by using IL-2-dependent CTLL cells. IFN-γ was assayed in a mouse IFN-γ ELISA test kit according to the procedure recommended by the manufacturer (Holland Biotechnology, Leiden, The Netherlands). Levels of IFN-γ were expressed in NIH U/ml.

Delayed type hypersensitivity assay. Viable T cells were harvested 7 days after stimulation with Ag and resuspended to the required concentration in PBS. T cells (2×10^5) together with 3 μg of UV-inactivated MV or Vero Ag were injected s.c. in a volume of 15 μl into the right or left ear, respectively, of naive female BALB/c mice ($n = 3$). Additional controls included mice injected with either of the Ag in the absence of T cells or in the presence of cloned RV-specific murine T cells with the $CD4^+$, $CD8^-$ phenotype (H.Bunschoten, B. Dietzschold, I. J. Th. M. Claassen, R. J. Klapmuts, F. G. C. M. Uytdehaag, and A. D. M. E. Osterhaus, manuscript in preparation). Ear thickness was measured at 24, 48, and 72 h by using a micrometer (Mitutoyo, Tokyo, Japan). Ear swelling was expressed in micrometers, as the mean value ± SD measured in the three mice per group. Background swelling responses of the control mice, which had received Ag in the absence of T cells, were subtracted from the responses of the animals which had received the T cells.

RESULTS

Generation of T cell clones. Spleen cells of mice primed with a homogenate of MV-infected mouse brain were isolated and subsequently stimulated in vitro with 0.5 or 20 μg of purified UV-irradiated MV per 1.5×10^6 cells. Lymphoblasts were isolated and subsequently cloned by limiting dilution. Densities of 100 and 500 cells/well resulted 10 to 14 days later in cell proliferation in 1 to 11% of the wells. On the basis of a calculation using the Poisson distribution, greater than 97% of the growing T cells were considered clonal. The T cell clones were maintained in culture by weekly restimulation with fresh irradiated spleen cells and UV-irradiated MV. fusionrom the spleens of three mice, 37 stable T cell clones were isolated and subsequently characterized. All T cell clones revealed a homogeneous surface phenotype, characteristic of murine Th cells, i.e., Thy 1.2^+, $CD4^+$, and $CD8^-$ (17).

Specificity of T cell clones. All stable T cell clones were tested for their MV and MV protein specificity in a T cell proliferative assay. Although all clones proliferated in the presence of MV and syngeneic APC, but not in the presence of Vero cell lysate, the protein specificity of

seven of the T cell clones could be estimated by culturing the cells in the presence of one of the purified viral protein preparations. The specificity of these and previously described T cells (0.5 E10, 20 F1, and 20 F3) is shown in Table I. T cell clones 1 0.5 C1 and 2 20 B9 and the previously described clones were shown to be specific for the fusion protein, clones 3 0.5 A2 and 3 20 B1 were specific for the H protein, and clones 2 20 A5, 2 20 C10, and 2 20 B10 responded to the N/M preparation. Ag recognition for all T cell clones seemed to be MHC-restricted at H-2^d, inasmuch as spleen cells from both the syngeneic BALB/c mice and the B10.D2 (H-2^d) mice did stimulate T cell proliferation in the presence of MV, whereas spleen cells from BALB/b (H-2^b) or C57BL (H-2^b) mice did not (data not shown).

The fine specificities of these fusion-specific T cell clones, together with two T cell clones (MV2 H10 and fusion3 A1) generated from fusion iscom-immunized mice, were further analyzed with β-galactosidase fusion proteins, which consisted of both the β-galactosidase protein and deletion products of the fusion protein. Six of the seven fusion-specific T cell clones (1 0.5 C1, 0.5 E10, 20 F1, 20 F3, MV2 H10, and F3 A1) proliferated in the presence of the β-galactosidase fusion protein, which contained the major part of the MV-F protein, i.e., amino acid 2-513, but not in the presence of only the β-galactosidase protein (Table II). These T cell clones and the H-specific T cell clone, 3 20 B1, were further tested with the panel of β-galactosidase fusion proteins, containing larger deletions in the fusion protein (Table II). T cell clones 20 F1, 20 F3, and MV2 H10 did not react with the fusion proteins containing amino acid 2-329 of the fusion protein. Two of these T cell clones (20 F1 and MV2 H10) were shown to recognize the MB70 fusion protein containing amino acid 428-495 of the fusion protein, whereas T cell clone 20 F3 did not proliferate significantly in the presence of this fusion protein. T cell clones 1 0.5 C1, 0.5 E10, and F3 A1 recognized the fusion protein containing amino acid 2-306 of the fusion protein. From these experiments it can be concluded that the region corresponding to amino acid 2-513 of the fusion protein contained at least two different epitopes recognizable by mouse T cells.

In vitro B cell stimulation by T cell clones. Selected T cell clones specific for the F, the H, or the N/M preparations (1 0.5 C1, 3 20 A2, or 2 20 A5, respectively), were examined for their ability to provide help to MV-specific B cells in vitro. In vivo MV-primed spleen cells depleted of T cells (hereafter referred to as B cells) were co-cultivated with different numbers of T cells in the presence of 7 ng of UV-inactivated MV, which was found to be the optimal dose of Ag for stimulation of MV-specific B cell responses under conditions described (data not shown). The F-specific T cell clone 1 0.5 C1 was shown to provide help to MV-specific B cells with as few as 100 T cells/5 × 10^5 B cells present in culture, as was demonstrated in both MV-ELISA and MV-HI assay (Fig. 1). In contrast, the N/M-specific T cell clone 2 20 A5 only induced MV-specific antibody responses, when 1000 or more than 1000 T cells/well were added to B cell cultures. The H-specific T cell clone 3 20 A2 only provided help when 10,000 or more T cells per well were added to the B cells (data not shown). Control cultures, in which Vero Ag was used as stimulating Ag, never contained MV-specific antibodies.

To investigate whether the T cell clone 1 0.5 C1 provided help to MV-primed B cells only, T cells were co-cultivated with a mixture of MV-primed and RV-primed B cells in the presence of either MV, RV, or a mixture of both Ag. Supernatants of these cultures were assayed for the presence of MV- and RV-specific antibodies. As shown in Figure 2, the mixture of B cells cultured in the presence of MV and T cell clone 1 0.5 C1 produced antibody to MV but not to RV. RV-specific antibodies were only produced, when in addition to MV and T cells, RV was also present. Because the $CD4^+$, $CD8^-$ T cell clones were shown to possess an in vitro helper function, the supernatants of these clones were analyzed 18 h after stimulation for the presence of IL-2 and IFN-γ. All of the clones tested produced IL-2 and IFN-γ upon stimulation with Ag (Table III).

DTH response induced by T cell clones. Adoptive transfer of T cells with an in vitro helper function was carried out to demonstrate that these T cell clones were able to induce a MV-specific DTH response. Three F-specific T cell clones, 1 0.5 C1, 20F1, and 0.5 E10, the H-specific T cell clone 3 0.5 A2, and the N or M protein-specific T cell clone 2 20 A5, were injected together with UV-inactivated MV or Vero cell Ag into the ears of naive syngeneic mice. All these clones were shown to induce MV-specific swelling of the ears measured at 24 h, whereas the RV-specific T cell clone AA8A failed to in-

TABLE I

Ag specificity of the response of T cell clones obtained from CAM/MBH-immunized mice[a]

T Cell Clones	Ag Used for Stimulation				
	MV	F	H	N/M	Vero cells
1 0.5 C1	37,401 ± 5,461	31,637 ± 3,117	1,235 ± 612	1,310 ± 155	914 ± 298
2 20 B9	2,963 ± 452	1,500 ± 175	68 ± 34	614 ± 129	114 ± 69
0.5 E10[b]	6,529 ± 242	824 ± 127	416 ± 115	198 ± 49	249 ± 91
20 F1[b]	5,375 ± 516	2,679 ± 249	166 ± 69	143 ± 13	117 ± 53
20 F3[b]	5,710 ± 1,930	2,505 ± 390	771 ± 435	1,175 ± 147	327 ± 218
3 0.5 A2	7,281 ± 862	229 ± 232	2,451 ± 251	154 ± 41	273 ± 210
3 20 B1	8,247 ± 1,939	161 ± 80	7,250 ± 3,854	283 ± 80	378 ± 144
2 20 A5	8,311 ± 502	281 ± 136	360 ± 380	2,038 ± 448	220 ± 102
2 20 C10	3,219 ± 311	257 ± 161	190 ± 145	1,489 ± 173	91 ± 41
2 20 B10	2,574 ± 307	122 ± 114	541 ± 199	1,386 ± 243	404 ± 71

[a] Cells (1 × 10^4) from T cell clones were co-cultured with 3 × 10^5 irradiated syngeneic spleen cells, incubated with MV, purified F protein or H protein, a mixture of N and M proteins, or Vero Ag. Stimulation was assessed 3 days later by measuring the incorporation of [^{3}H]thymidine added 18 h before termination of the cultures. Results are expressed as mean cpm ± SD of triplicate cultures.

[b] T cell clones previously described by de Vries et al. (6).

TABLE II

Specificity of the response of F-specific T cell clones to β-galactosidase fusion proteins, containing different parts of the F protein[a]

T Cell Clones	MV	Vero Cell	β-Galactosidase Fusion Proteins (aa)				
			pEX3 (-)	MB70 (428-495)	30 (2-306)	T2h (2-329)	117 (2-513)
1 0.5 C1	15.278 ± 4.227	272 ± 102	784 ± 115	1.509 ± 77	19.945 ± 1.470	19.947 ± 1.847	14.851 ± 1.200
0.5 E10	6.798 ± 1.148	137 ± 20	443 ± 63	520 ± 61	3.339 ± 268	3.998 ± 389	3.851 ± 716
F3 A1	4.601 ± 836	225 ± 36	1.774 ± 125	1.828 ± 191	7.010 ± 422	7.050 ± 233	6.485 ± 246
20 F1	6.148 ± 823	179 ± 68	433 ± 27	6.526 ± 749	928 ± 52	955 ± 96	11.999 ± 701
20 F3	7.619 ± 735	516 ± 45	1.009 ± 138	2.085 ± 278	NT[b]	1.792 ± 105	6.459 ± 1.450
MV2 H10	14.772 ± 1.814	871 ± 483	988 ± 236	7.746 ± 922	1.322 ± 177	1.504 ± 308	12.561 ± 852
2 20 B9	2.617 ± 417	355 ± 6	732 ± 79	NT	NT	NT	847 ± 53
3 20 B1	11.812 ± 1.525	566 ± 169	754 ± 91	1.505 ± 300	404 ± 86	1.833 ± 276	1.470 ± 110

[a] Cells (1×10^4) from F-specific T cell clones were co-cultured with 5×10^5 irradiated syngeneic spleen cells and incubated with β-galactosidase F proteins containing different parts of the F protein, as is indicated for each protein by the amino acid numbers (aa). Stimulation was assessed as described in the legend of Figure 1. Results are expressed as mean cpm ± SD of triplicate cultures.

[b] NT, not tested.

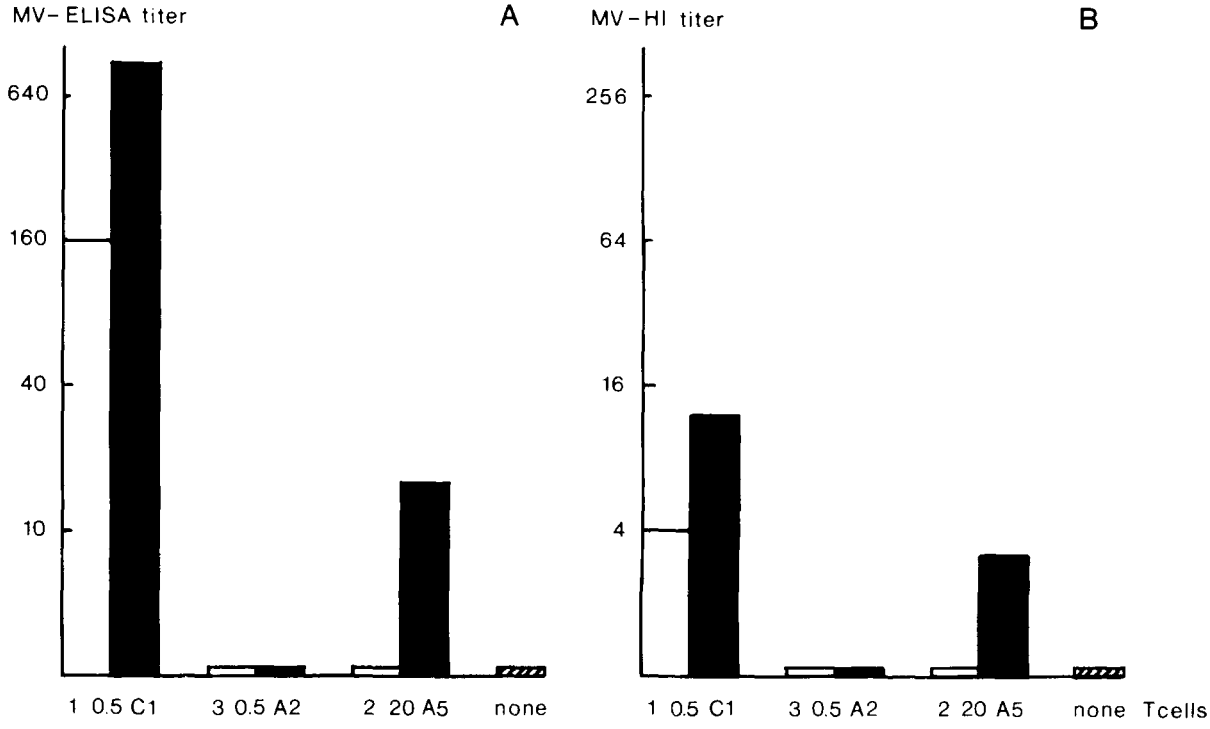

Figure 1. In vitro helper function of F, H, or M/N protein-specific T cell clones. Spleen cells from MV-immunized mice, depleted of T cells by anti-Thy 1.2 and C, were cultured at 5×10^5 cells/well with 100 (□) or 1000 (■) T cells/well of the clones 1 0.5 C1, 3 0.5 A2, or 2 20 A5, or without T cells (▨) and in the presence of 7 ng of UV-inactivated MV/well for 7 days. Thereafter the supernatants were harvested and assayed in MV-ELISA (*A*) and in MV-HI assay (*B*). The results represent the mean antibody titer of supernatants obtained from triplicate wells.

duce MV-specific swelling (Fig. 3). At 48 h, ear swelling reactions had decreased but were still detectable (data not shown).

DISCUSSION

T cell clones that reacted specifically with MV were obtained from mice immunized with MV-infected mouse brain homogenate. Although no MV-specific T cell proliferation was observed in the bulk spleen cell cultures of these mice (P. de Vries and I. K. G. Visser, unpublished observation), cloning by limiting dilution of lymphoblastoid cells after in vitro stimulation with UV-inactivated MV resulted in the generation of 37 stable MV-specific T cell clones. On basis of the low frequency of proliferating T cells, the generated T cells were considered clonal.

All the T cell clones recognized Ag in the context of H-2^d molecules and displayed a helper phenotype (Thy-1.2^+, CD4^+, CD8^-). T cell clones with a Thy-1.2^+, CD4^-, CD8^+ phenotype were not established by the cloning procedure used, possibly for the following reasons: 1) the MV-infected brain homogenate from newborn mice used for in vivo immunization may not induce MHC class I-restricted CD8^+ T cells, inasmuch as no viral replication will occur in adult mice after i.p. inoculation; 2) MHC class I-restricted CD8^+ T cells may have been generated in vivo against MV-infected brain homogenate, but the repeated in vitro stimulation with UV-inactivated MV may have selected for T cell clones bearing the helper phenotype (Thy-1.2^+, CD4^+, CD8^-). Recently, Morrison et al. (18) have shown that the Ag form influences the induction and frequency of influenza virus-specific class I and class II MHC-restricted T lymphocytes. Experiments in which syngeneic stimulator and/or target cells infected, e.g., with vaccinia virus recombinants encoding either the H, F, or internal proteins will eventually enable the generation and characterization of MV-specific T cells bearing

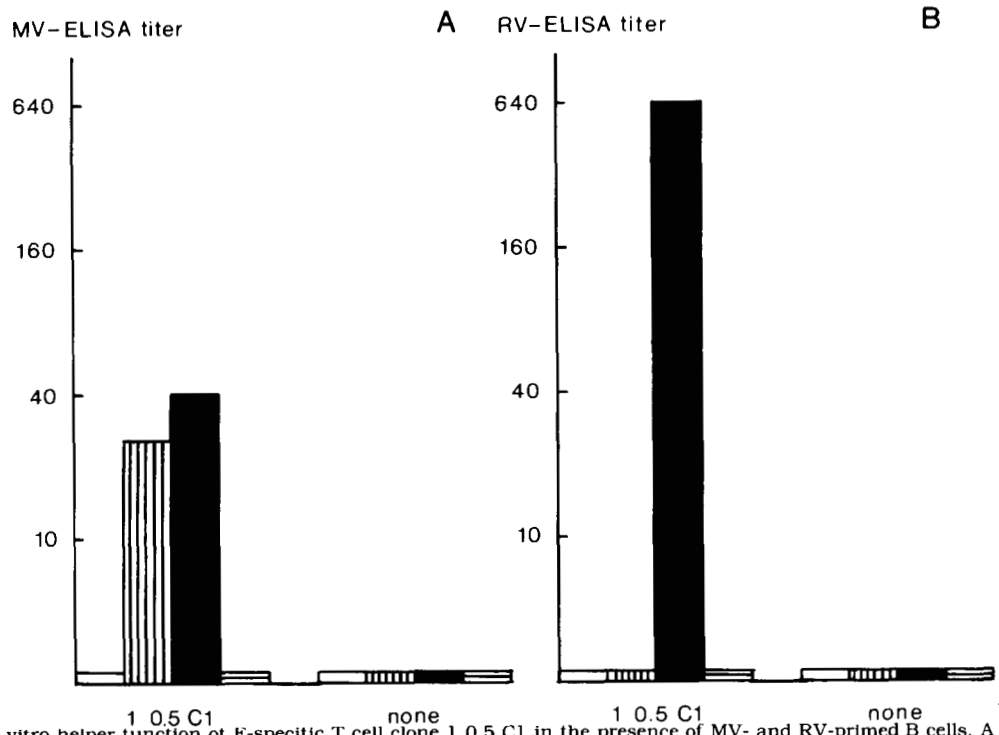

Figure 2. In vitro helper function of F-specific T cell clone 1 0.5 C1 in the presence of MV- and RV-primed B cells. A mixture of MV- and RV-primed spleen cells, both depleted of T cells by anti-Thy-1.2 and C, was cultured at 5×10^5 cells/well with 100 cells/well of T cell clone 1 0.5 C1 or no T cells in the absence (□) or presence of 7 ng of MV (▥), 1 μg of RV (▩) or both 7 ng of MV and 1 μg of RV (■) as stimulating Ag for 7 days. Thereafter the supernatants were harvested and assayed in MV-ELISA (*A*) and RV-ELISA (B). The results represent the mean antibody titer of supernatants obtained from triplicate wells.

TABLE III

IL-2 and IFN-γ production by T cell clones 1 0.5 C1, 3 20 A2, and 2 20 A5[a]

T cell clone	Ag	IL-2 (U/ml)	IFN-γ (U/ml)
1 0.5 C1	MV	20.00	540
2 20 A5	MV	4.00	300
3 0.5 A2	MV	3.80	650
1 0.5 C1	Vero	<1.00	<2

[a] Cells (1×10^4) were co-cultured with 5×10^5 irradiated syngeneic spleen cells incubated with UV-inactivated MV or Vero Ag. Supernatants were assayed 18 h after stimulation for the presence of IL-2 and IFN-γ as described in Materials and Methods.

the $CD4^-$, $CD8^+$ phenotype.

The protein specificity of 7 of the 37 MV-specific T cell clones could be assessed by measuring proliferative responses: T cell clones specific for the fusion protein (clones 1 0.5 C1, 2 20 B9), the H protein (clones 3 0.5 A2 and 3 20 B1) and N/M preparation (clones 2 20 A5, 2 20 C10, and 2 20 B10) were generated. The inability to define the polypeptide specificity of the other clones by this method may have been caused by differences in uptake, processing, and presentation of proteins presented either in a multimeric form (UV-inactivated MV), or in a purified form. Furthermore, certain T cell clones may only recognize Ag in a conformation-dependent way and others may recognize other than the purified MV proteins used.

The F-specific T cell clones were, together with the T cell clones previously generated (0.5 E10, 20 F1, 20 F3, MV2 H10, and F3 A1), characterized for their fine Ag specificities. With use of four β-galactosidase fusion proteins, which contained different parts of the fusion protein, we showed that at least two epitopes recognizable by murine T cells are present on the fusion protein. The first 306 amino acids of the fusion protein, corresponding

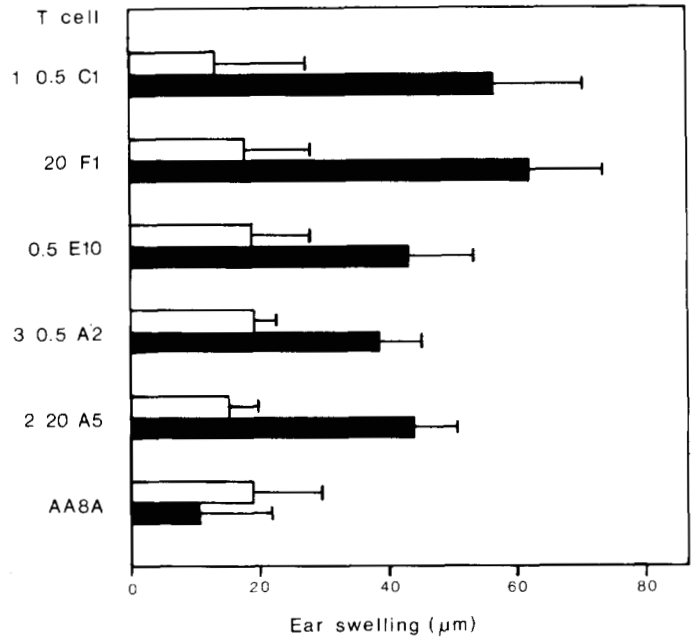

Figure 3. DTH response in naive mice induced by MV-specific T cells. T cells (2×10^5) of clones 1 0.5 C1, 20 F1, 0.5 E10, 3 0.5 A2, and 2 20 A5, and of a RV-specific T cell clone (AA8A) were injected into the ears of naive, syngeneic mice in the presence of 3 μg of UV-inactivated MV (■) or Vero Ag (□). Ear swelling was measured at 24 h and is expressed in micrometers as the mean value ± SD of independent measurements of three mice within each group. The mean swelling of ears injected with Ag alone was first subtracted.

to the F_2 component and the N-terminal part of the F_1 component, harbors at least one epitope, and the region of amino acid 428-495 of the C-terminal part of the F_1 component contains at least one other epitope.

The functional analysis of the T cells with a $CD4^+$,

$CD8^-$ helper phenotype showed that the clones 1 0.5 C1 and 2 20 A5 provided help to MV-specific B cells in vitro in the presence of MV Ag. MV-specific antibodies could be measured in MV-ELISA and in MV-HI assay. This showed that both the F- and the N- or M protein-specific T cells were able to provide help in the production of biologically active antibodies directed against the H protein. Although clone 3 0.5 A2 did not exert helper function under similar conditions, it did stimulate MV-specific antibody production for increasing numbers of T cells present in culture (data not shown). Recently, it has become evident that mouse T cells with a helper phenotype are heterogeneous with regard to their lymphokine production and can be divided accordingly into two subsets (19). T_H1 cells synthesize IL-2 and IFN-γ but not IL-4 and IL-5, whereas T_H2 cells produce IL-4 and IL-5 but not IL-2 and IFN-γ. Our observation that all three T cell clones produce IL-2 and IFN-γ in response to stimulation with UV-inactivated MV (Table III) suggests that they belong to the T_H1 helper subset. In line with the evidence suggesting that B cell activation by T_H1 is a lymphokine-mediated event not requiring cognate interactions, we observed that T cell clone 1 0.5 C1, when cultured in the presence of both MV- and RV-specific B cells and both Ag, activated MV-specific B cells as well as RV-specific "bystander" B cells (20). Furthermore, it has been shown that T_H1 cells may be involved in a DTH response (21). All MV-specific T cell clones tested proved able to induce a MV-specific DTH response. Whether the $CD4^+$, $CD8^-$ T cell clones also possessed cytolytic activity could thus far only be investigated by using murine target cells pulsed with MV Ag. None of our T cell clones tested was able to induce more than 10% of specific lysis of MV-pulsed target cells (data not shown). Experiments in which target cells infected, e.g., with vaccinia virus recombinants encoding the MV proteins are used, are in progress to study the potential of the $CD4^+$, $CD8^-$ T cell clones in a cytolytic assay in more detail.

In the present paper we investigated whether T cell clones, specific for the F protein, which displayed the $Thy1^+$, $CD8^-$, $CD4^+$ helper phenotype and were shown to belong to the T_H1 subset of murine T cells, play a role in protection against MV infection by adoptive transfer of clones in a mouse challenge model. The relevance of our studies in the mouse model for the human immune response to MV infection is not clear. However, our recent findings suggest that human $CD4^+$, $CD8^-$ MHC class II-restricted T cell clones recognize epitopes on regions of the F protein, where epitopes recognized by murine T cell clones also are present.

Acknowledgment. We thank Ms. C. Kruyssen for preparing the manuscript.

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