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The immunosuppressive effects of measles virus on T cell function—failure to affect IL-2 release or cytotoxic T cell activity *in vitro*

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SUMMARY

Measles virus (MV) is known to depress T cell function. In order to determine whether this results from alteration in the production of, or response to, interleukin-2 (IL-2) we studied the effect of *in vitro* infection with MV on human IL-2-dependent T cell lines. MV produced a cytopathic productive infection in these cells. Class I allospecific cytotoxic T cells retained their cytotoxic activity 48 h after infection. Both cytotoxic and Leu 3a/4a positive T cell lines continued to respond to IL-2 by proliferation up to 26 h after infection. The ability of human tonsillar lymphocytes to generate IL-2 in response to phytohaemagglutinin following MV infection was then studied. In early measles infection (up to 48 h) there was no suppression of IL-2 production: in fact measles infected cells spontaneously released low levels of IL-2 in the absence of lectin. Similarly, IL-2 release was not affected by *Herpes simplex* virus infection of such cultures, although lymphocytes infected with Sendai or respiratory syncitial viruses produced considerably less IL-2. These observations suggest that MV-induced immunosuppression is not a result of inhibition of differentiated T cell function, IL-2 generation or responsiveness, but may be more directly related to virus-induced cytopathic effects in activated T cells.

Keywords measles virus interleukin-2 cytotoxic T cell

INTRODUCTION

Measles virus (MV) is a paramyxovirus which besides producing the characteristic exanthem may also produce persistent infection and progressive neurological disease—subacute sclerosing panencephalitis (Morgan & Rapp, 1977). Many different cell types may be infected with this virus (Matumoto, 1966) including human lymphocytes (Sullivan *et al.*, 1975a; Huddlestone, Lampert & Oldstone, 1980). Following natural infection MV may be recovered from peripheral blood lymphocytes (PBL), particularly T cells (Whittle *et al.*, 1978).

It was long ago observed that the mantoux test is reduced during and following acute measles infection (von Pirquet, 1908). This cutaneous anergy is one manifestation of the reduction in cell-mediated immunity which follows MV infection and which has been implicated in the high mortality associated with MV infection in developing countries (Whittle *et al.*, 1973; Morday, 1969).

These clinical observations have prompted numerous studies of T cell responses following their *in vitro* infection with MV. Proliferative responses to phytohaemagglutinin (PHA) are reduced

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following *in vitro* infection (Sullivan *et al.*, 1975b; Lucas, Galama & Ubels-Postma, 1977), but variable responses are observed following *in vivo* infection or live virus vaccination (Whittle *et al.*, 1973). Specific proliferative responses to MV antigens are found in normal seropositive subjects (McFarland *et al.*, 1980). Galama *et al.* (1980) examined the effect of measles virus infection *in vitro* on the allogeneic mixed lymphocyte culture (MLC). They observed that the acquisition of cytotoxic function could be impaired by infection on day 1 of the culture, but was unimpaired if the virus was added on day 5. The proliferation of antigen or lectin activated T cells (Smith & Ruscetti, 1981) is now known to depend on T cell growth factor (TCGF) or interleukin-2 (IL-2), which is itself produced by activated T cells. As MV infects T cells, one possible explanation for reduced PHA proliferation and acquisition of cytotoxic T cell (Tc) activity in the MLC, and for the observations *in vivo*, could be that IL-2 production or responsiveness is reduced following MV infection.

In order to test this hypothesis we have examined the effect of measles virus on IL-2 production, and responses to it, by T cells. Here we report that measles virus infected IL-2-dependent Tc, and Leu $3a^+$ T cell lines, continue to respond to IL-2 and remain cytotoxic. MV infection also does not impair lectin stimulated IL-2 production until 48 h following infection. These observations suggest that MV-induced immunosuppression is unlikely to be mediated by altering IL-2 responses.

MATERIALS AND METHODS

Viruses. Edmonston strain measles virus was propagated in Vero cells to a titre of 2×10^7 pfu/ml. The virus was inactivated by u.v. light to a residual titre of $< 10^4$ pfu/ml and by β-propiolactone. Sendai virus (10^8 pfu/ml), respiratory syncitial virus (RSV) (2×10^6 pfu/ml) and PR8 influenza virus were gifts from Dr K. Apostolov. *Herpes simplex* type 1 (HSV 1) (2×10^7 pfu/ml) was kindly supplied by C. Lancashire.

Cell culture and media. RPMI 1640 (Flow) supplemented with 10% fetal calf serum (FCS), 2 mM/l L-glutamine, penicillin 100,000 iu/l and streptomycin 100 mg/l was used in all experiments unless otherwise stated.

TcL1 is an IL-2-dependent cytotoxic T cell line established in a one way MLC and maintained for 45 days prior to these experiments. Its surface phenotype is 95% Leu $4a^+$ (pan-T), 98% Leu $2a^+$ (cytotoxic/suppressor), 2% Leu $3a^+$ (helper/inducer) and 70–80% DR^+. The lytic specificity was against class I HLA antigens A7, A28, B7 and B44—when assayed against a panel of PHA lymphoblasts—with no NK activity against K-562 cells.

Tpha were PHA blasts produced by stimulating Ficoll-Hypaque isolated PBL with 2 μg/ml purified PHA (Sigma). Blasts were isolated after 5 days and maintained in IL-2 for a further 18 days prior to use; their surface phenotype was 90% Leu 4a and 93% Leu 3a positive.

Cytotoxicity assays. PBL were isolated by Ficoll-Hypaque density centrifugation from subjects of known HLA type, both susceptible and non-susceptible to lysis by TcL1. These cells were incubated with PHA containing medium (2 μg/ml) for 48 h. The cells were labelled with ^{51}Cr (Amersham) at 200 μCi/10^6 cells. They were washed and suspended to 5×10^4 cells/ml and 100 μl were incubated, in triplicate, with varying numbers of TcL1 in a total volume of 200 μl for 6 h. Specific release was estimated as previously described (Borysiewicz *et al.*, 1983).

IL-2 production and assay. Lectin free conditioned medium, containing IL-2, for maintaining T cell lines was produced as described (Borysiewicz *et al.*, 1983). In experiments where the effect of virus on IL-2 production was examined, the lymphocytes were pulsed with 10 μg/ml PHA for 2 h and then washed free of PHA before being incubated as described. IL-2 activity was assayed in these experiments using 6 day PHA blasts. One hundred thousand PHA blasts per well of a 96 well flat bottomed plate (Costar) were incubated in triplicate with serial $\log_2$ dilutions of the u.v. irradiated sample to be assayed. After 20 h incubation at 37°C, 1 μCi ^3H-thymidine (Amersham) was added to each well for a further 6 h. The samples were harvested with a Titertek harvester and ^3H-thymidine incorporation estimated. Where appropriate the samples were also assayed on PBL to determine the presence or absence of PHA.

Antibodies. Commercial anti-human T cell monoclonal antibodies, anti-Leu 4a, 2a, 3a and anti-DR (Becton-Dickson), were used according to the manufacturer's protocols. MV infected cells

Table 1. Infection of TcL1 and 6 day PHA blasts from two subjects with Edmonston strain measles virus

	Time post-infection (h)	Cell number*		% SSPE positive cells†	
		Measles infected	Uninfected	Measles infected	Uninfected
TcL1	48	61%	142%	71%	0%
	24	67%	135%	57%	1%
	8	87%	—	65%	0%
6 day PHA blasts (1)	48	50%	ND	82%	8%
	24	71%	ND	54%	6%
	8	95%	ND	52%	7·5%
6 day PHA blasts (2)	48	44%	ND	69%	10%
	24	53%	ND	40%	7%
	8	96%	ND	60%	9%

ND = not done.

* Cell number expressed as number of viable cells recovered/number of cells originally infected × 100.

† % of lymphocytes expressing positive indirect immunofluorescence with SSPE serum.

were identified by indirect immunofluorescence using serum obtained from a patient with subacute sclerosing panencephalitis and commercial sheep anti-human Ig (Wellcome). Cell viability in all experiments was assessed by ethidium bromide (5 μg/ml) and acridine orange (5 μg/ml) staining.

RESULTS

MV infection in IL-2-dependent T cell lines

TcL1 and 6 day PHA blasts were infected with live MV at a multiplicity of infection (MOI) of 5:1 for 2 h and then cultured in the presence of optimal concentrations of lectin free IL-2. These cells

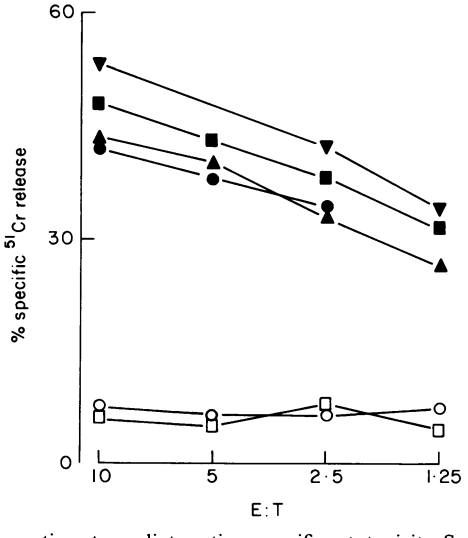

Fig. 1. MV infected TcL1 cells continue to mediate antigen specific cytotoxicity. Susceptible target cells (●, ▲, ■, ▼) were lysed by TcL1 cells infected with measles virus for o (●), 2 (▲), 24 (▼) and 48 h (■) in a 6 h ^{51}Cr release assay. There was no lysis of previously non-susceptible (○, □) target cells.

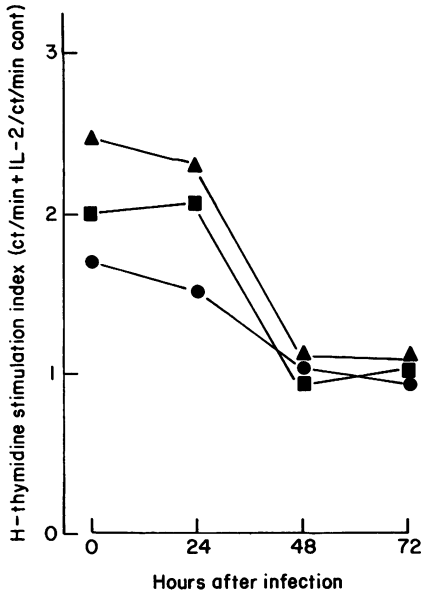

Fig. 2. MV infected TcL1 (●) and 6 day PHA blasts (▲, ■) continue to respond by proliferation to IL-2 up to 26 h post-infection. Viable lymphocytes infected with measles virus (48, 24 and 2 h previously) and maintained in optimal concentrations of IL-2 were harvested, and 2×10^4 viable cells placed in medium alone or medium supplemented with fresh IL-2 for a further 20 h. ^{3}H-thymidine incorporation after a 4 h pulse was estimated and results expressed as a stimulation index (ct/min in IL-2 culture/ct/min in absence of IL-2).

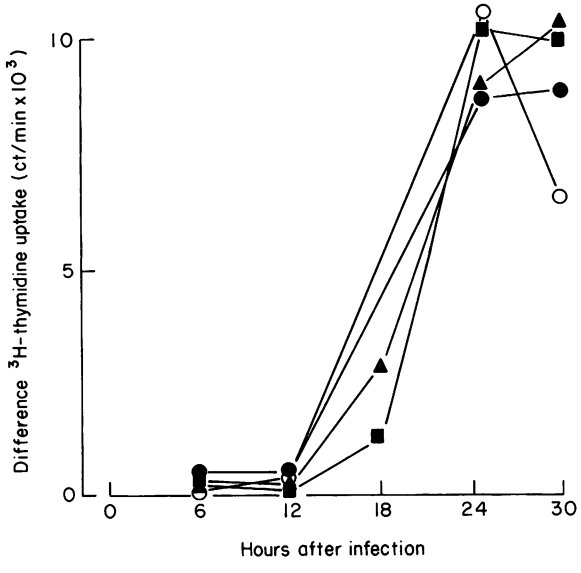

Fig. 3. Tpha (Leu 3a phenotype) proliferate in response to IL-2 up to 30 h following MV infection. Tpha were uninfected (○), infected with live (●), u.v. inactivated (▲) or β-propiolactone inactivated (■) MV and cultured in presence or absence of IL-2. Proliferation was estimated by a 4 h pulse of ^{3}H-thymidine and results expressed as the difference in ^{3}H-thymidine uptake between IL-2 supplemented and medium alone cultures. At 30 h > 90% of lymphocytes were viable in all cultures and only live MV infected cells expressed measles surface antigens (60%).

were infected with MV (Table 1). Infection was lytic as indicated by the falling cell numbers, and was productive as MV was isolated from the culture supernatants (data not shown).

MV does not abrogate Tc function

TcL1 was infected with live MV as described above. After infection for 2, 24 and 48 h non-viable cells (as identified by ethidium bromide) were removed by Ficoll-Hypaque centrifugation. Cytotoxic activity of viable cells was assayed in a 6 h ^{51}Cr release assay against susceptible and resistant PHA blasts. There was no reduction in cytotoxicity although 60% of the viable cells at 24 and 48 h post-infection expressed MV cell surface antigens (Fig. 1).

Effect of MV on IL-2 responsiveness of T cell lines

As the cytotoxic effector function of TcL1 was not affected by the presence of replicating MV it seemed possible that the reduced activity of Tc in MLC cultures could be explained by a reduction in IL-2 responsiveness. To test this hypothesis, viable TcL1 cells were infected with measles virus for 2 h and then maintained in IL-2. Twenty-four hours prior to assay the lymphocytes were washed and cultured either in an optimal concentration of IL-2 or in medium alone for a further 20 h. ^{3}H-thymidine was then added to the culture and incorporation estimated after 4 h. These lymphocytes responded to IL-2 by increased ^{3}H-thymidine uptake up to 26 h post-infection (Fig. 2).

These observations were confirmed using Tpha incubated for 2 h with live, u.v. and β-propiolactone inactivated virus, washed free of virus and cultured in the presence or absence of exogenous IL-2. At varying times following infection (2, 8, 14, 20 and 26 h) ^{3}H-thymidine (1 μCi/well) was added and the cells harvested 4 h later. There was no significant difference in ^{3}H-thymidine incorporation, up to 30 h post-infection (Fig. 3) when cell viability was >90% and 60% of cells infected with live virus expressed measles surface antigens.

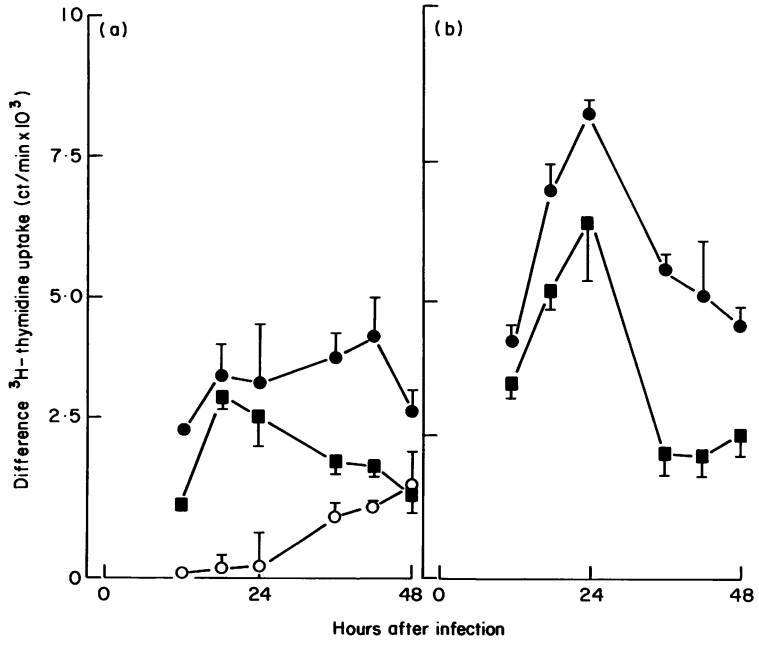

Fig. 4. Measles virus infection does not inhibit IL-2 production in PHA stimulated cultures. Tonsillar lymphocytes were pre-treated with PHA (a) and infected with live MV (MOI 5:1) or infected and then pulsed with PHA (b). Supernatants from cultures were harvested at various times and assayed on 6 day PHA blasts as described in Materials and Methods. Lymphocytes infected with measles in the presence of PHA (●——●) released IL-2 in similar amounts to uninfected PHA pulsed lymphocytes (■——■). Cells infected with MV in the absence of PHA (○——○) produced low levels of IL-2.

Table 2. Proliferative response and IL-2 release of tonsillar lymphocytes 48 h following PHA stimulation and virus infection

Virus*	PHA	Proliferation response ct/min (s.d.)	IL-2 release† stimulation index (s.d.)
No virus	–	1,189 (70)	3·75 (0·6)
No virus	+	11,731 (780)	7·8 (0·4)
Sendai	–	104 (52)	1·75 (1·0)
Sendai	+	534 (630)	3·7 (1·0)
RSV	–	745 (125)	0 (—)
RSV	+	437 (17)	0 (—)
PR8	–	823 (311)	2·75
PR8	+	4,937 (737)	5·5
HSV	–	895 (160)	2·25
HSV	+	9,974 (833)	5·5

* MOI 2:1.

† IL-2 assayed on 6 day PHA blasts as described in Materials and Methods.

MV does not inhibit IL-2 production by lectin stimulated T cells

The reduced PHA and MLC proliferative responses might also have been explained by a reduction in IL-2 production following MV infection. In order to investigate this possibility tonsillar lymphocytes were prepared by disruption of tonsils and Ficoll-Hypaque isolation of released lymphocytes. These cells were infected with MV for 2 h either preceding or following a 2 h pulse of PHA. The lymphocytes were washed free of virus and PHA, suspended at 2×10^6/ml and incubated for varying periods. During the 48 h of the experiment there was no significant suppression of IL-2 production (Fig. 4) and low levels of IL-2 activity were produced by MV infected cells in the absence of PHA stimulation.

Effect of infection with other related and unrelated viruses on IL-2 production

The effect of Sendai virus, RSV, HSV and PR8 influenza virus on PHA-induced proliferation and IL-2 release was examined. Tonsillar lymphocytes were pulsed with PHA or cultured in medium and then incubated for 2 h with live or heat-inactivated (56°C for 2 h) virus at an MOI of 2:1. The cells were washed free of virus and incubated at 2×10^5 cells/200 μl in round bottomed microtitre plates. After 48 h 100 μl of supernatant was removed for assay of IL-2 activity and proliferation measured in the remaining cells by ^{3}H-thymidine incorporation. Viability of lymphocytes was $> 82\%$ in the virus infected cultures although giant cells were seen in measles and RSV infected cultures.

Lymphocytes treated with heat-inactivated virus proliferated and released IL-2 as did uninfected cultures. IL-2 release was markedly reduced by Sendai and RSV infection, but influenza and HSV only partly reduced IL-2 release (Table 2).

DISCUSSION

These studies were designed to determine whether MV infection of T cells induced alteration in the production of, or responsiveness to, IL-2. They show that within the first 48 h of MV infection (1) an infected alloreactive T cell line does not lose its specific cytotoxicity (2) infected established T cell lines are still capable of proliferating in response to IL-2 (3) production of IL-2 by infected PHA stimulated lymphocytes is unimpaired. Finally it was observed that (4) MV infection of lymphocytes in itself results in production of detectable amounts of IL-2.

It has long been recognised that patients with measles have impaired cellular immunity associated with *in vivo* infection of all types of lymphocytes (Whittle *et al.*, 1978). Sullivan *et al.* (1975a) showed that lymphocytes could be productively infected with measles *in vitro* and several studies have shown reduced lectin and MLR proliferative responses of these infected cells (Sullivan *et al.*, 1975b; Lucas *et al.*, 1977).

IL-2 is recognised as playing a pivotal role in T cell proliferative responses allowing the clonal expansion of antigen or lectin activated T cells, which express receptors for IL-2 on their cell surface (Smith & Ruscetti, 1981). The source of IL-2 may be from both T8 and T4 positive lymphocytes (Luger *et al.*, 1982), although the latter probably predominate.

It is known that measles virus may infect both types of cell (Huddlestone *et al.*, 1980) and it seemed reasonable to suppose that reduced IL-2 production could explain the loss of proliferative responses to PHA. Indeed it has already been suggested (Wainberg, Vydelingum & Margolese, 1983) that the non-specific suppression of lymphocyte proliferative responses, by a number of viruses that do not productively infect lymphocytes, may be due to altered production of IL-2.

Our data shows that no simple quantitative reduction of IL-2 production occurs following MV infection. Indeed live MV appeared to stimulate IL-2 release in the absence of lectin. This effect was not observed with heat-inactivated virus preparations suggesting that it was not due to antigen specific activation. It is possible that this effect is due to a direct 'lectin like' effect of MV but further studies are needed to determine the mechanism.

Measles was not the only virus which had little effect on IL-2 production during the early phase of viral infection: HSV and influenza also produced only partial inhibition of IL-2 release after 48 h. However, RSV and Sendai virus (also paramyxoviruses), profoundly inhibited production of IL-2 and lymphocyte proliferation. These effects could theoretically be mediated either by infection of the macrophages in the case of influenza, or by a direct effect on lymphocytes—Sendai and RSV (Roberts, 1982). This inhibition of IL-2 production does not depend on a productive infection—as neither influenza nor Sendai productively infect lymphocytes (Zisman & Denman, 1973)—and may be non-specific (Wainberg *et al.*, 1983). In contrast measles (Sullivan *et al.*, 1975a) and *Herpes simplex* (Rinaldo *et al.*, 1978) have been shown to productively infect lymphocytes, but as we show here IL-2 production remains unimpaired early in infection. This suggests that specific or non-specific suppression of IL-2 production does not explain the immunosuppressive effects of measles virus infection.

Our observations are not necessarily at variance with the previously observed reduced proliferation of measles infected lymphocytes in response to PHA. Most of these studies had involved estimation of ^{3}H-thymidine incorporation at 48 or 96 h after infection. At these times there may be reduction of viable cell number and reduced responsiveness to IL-2. Our data shows that MV did not reduce responsiveness to IL-2 until 26–48 h following infection. This suggests that MV does not reduce IL-2 receptor expression in the early stages of measles infection and subsequent lack of responsiveness may be related to the direct cytopathic effect of measles infection. We also extended the observations of Galama *et al.* (1980), as we have shown that allospecific cytotoxic T cell lines infected with measles for 48 h, when more than 60% of cells expressed measles antigens, still retained specific cytotoxicity.

In summary, these studies indicate that *in vitro* measles infection does not abrogate differentiated T cell function and does not inhibit IL-2 production or responsiveness to IL-2 until late in infection. This suggests that the immunosuppressive effects of MV require the infection to proceed to late stages with virus replication. This is supported by observations of Lucas *et al.* (1977) who showed that measles infected lymphocytes will proliferate in response to PHA if they are cultured at 39°C, a temperature that is relatively inhibitory to MV replication. Although it is possible that measles may inhibit antigen specific activation of T cells prior to any requirement for IL-2, the predominant mechanism of the suppression observed *in vivo* may be a result of direct cytopathic effects of MV rather than any more subtle effects early in MV infection.

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