Clin. exp. Immunol. (1984) **58**, 116–126.

Interferon production by cultured peripheral leucocytes of MS patients

GRETA VERVLIET, H. CARTON, E. MEULEPAS & A. BILLIAU *Departments of Brain & Behaviour and Human Biology, The Rega Institute, University of Leuven, Leuven, Belgium*

(Accepted for publication 27 April 1984)

SUMMARY

Peripheral blood leucocytes from multiple sclerosis (MS) patients and from normal individuals were tested for their interferon (IFN) producing capacity after stimulation *in vitro* with various lectins and viruses. The lectins, Con A, PHA and PWM, induced IFN-γ. In a kinetic study, the response to Con A revealed itself as an all or none event: the number of responding cultures increased with increasing mitogen dose, but the IFN yield in responding cultures did not differ significantly between dose levels. Thus, any patient or donor could easily be rated as a responder or non-responder. About 1/2 of the MS patients were found to be non-responders if Con A or PHA were used as stimuli. Ninety per cent of the normal donors on the other hand were responders. With PWM as a stimulus 100% of both the MS patients and normal donor groups were found to be responders. Also, with PWM very small doses were sufficient to obtain a 100% response rate among tested cultures, and IFN production persisted for 5 days, while with Con A or PHA it was arrested after 2–3 days. The results indicate that the MS associated lesion is not the absence of functional impairment of all IFN-γ producing cells, but in only a fraction of them or in an accessory cell population required for the response to Con A and PHA but not to PWM. Newcastle disease virus (NDV) and vesicular stomatitis virus (VSV) both induced IFN-α. With NDV as the inducer response rates were 100% and yields were high irrespective of whether the cells were derived from patients or control donors. In contrast, with VSV as the inducer lower response rates were found in cultures from MS patients than in those from controls.

Keywords interferon multiple sclerosis

INTRODUCTION

Several research groups have found that peripheral blood leucocytes (PBL) from some but not all multiple sclerosis (MS) patients have a diminished ability to produce interferon (IFN) after virus or mitogenic stimulation (Bloom, 1980; Neighbour, Miller & Bloom, 1981; Salonen *et al.*, 1982a, 1982b; Gyodi *et al.*, 1982; Vervliet *et al.*, 1983). Other investigators did not find such a defect (Santoli, Trinchieri & Koprowski, 1978; Santoli *et al.* 1981; Zander *et al.*, 1982). This apparent conflict is difficult to resolve because different studies were done with different IFN inducers and under different experimental conditions. In the present study PBL cultures of MS patients and normal blood donors were stimulated with a set of mitogens and viruses at different dose levels. The data reveal that whether or not MS patients are found to be defective in their IFN response depends on the stimulus used.

Correspondence: Dr Greta Vervliet, Department of Brain and Behaviour, Laboratory of Neurochemistry, Campus Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium.

MATERIALS AND METHODS

Patients and normal donors. The criteria used in the diagnosis of MS were those of Rose *et al.* (1976). The degree of neurological impairment according to the disability status scale of Kurtzke varied between 3 and 8 (Kurtzke, 1970). None of the patients received corticosteroids or immunosuppressive therapy. Their age varied between 20 and 55 years. Healthy hospital personnel with ages varying from 25 to 45 years were used as normal controls.

IFN inducers. Concanavalin A (Con A, Calbiochem-Behring, La Jolla, California, USA) was used at final concentrations of 5, 10, 30 and 60 μg/ml; pokeweed mitogen (PWM, Serva Feinbiochemica, Heidelberg, Federal Republic of Germany) at 1·5, 5 and 10 μg/ml; phytohaemagglutinin (PHA, type V, Sigma, St Louis, Missouri, USA) at 10 μg/ml. Vesicular stomatitis virus (VSV) was used as inducer virus at a multiplicity of infection (moi) pf 0·1, 0·5 and 1·0 plaque forming units (PFU)/cell. VSV was grown in chick embryo fibroblast cultures for 24 h. The supernatant fluid was harvested and centrifuged (20 min, 3000 r/min). The titre of the VSV stock was $10^{8.2}$ PFU/ml on Vero cells. Newcastle disease virus (NDV) was used at a moi of 0·1 PFU/cell. The virus was grown by intra-allantoidal inoculation in 11 day old chick embryo's, incubated for 48 h. The supernatant was centrifuged (20 min, 3,000 r/min). The titre was $10^{7.6}$ PFU/ml on Vero cells.

IFN induction. Isolation of mononuclear cells from heparinized blood by Ficoll density gradient centrifugation was performed as described in a previous paper (Vervliet *et al.*, 1983). One millilitre aliquots of the cell suspension (2×10^6 cells/ml) were incubated in the presence of Con A (5, 10, 30 or 60 μg/ml); PWM (1·5, 5, 10 μg/ml) or PHA (10 μg/ml) and without inducer as a control for spontaneous IFN production. The culture supernatant fluids were harvested at 18 h by centrifugation at 500*g* for 10 min. All samples were stored at −20°C until further assay.

IFN assay. IFN assay and characterization studies were carried out as extensively described in a previous paper (Vervliet *et al.*, 1983).

RESULTS

IFN responses to Con A, PHA and PWM

PBL from normal donors and from MS patients were cultured without added inducer, with Con A, PWM or with PHA (10 μg/ml). After 24 h the supernatant fluids were harvested for IFN titration. As can be seen in Table 1, only 55% of the cultures from MS patients produced detectable IFN (i.e. $\geqslant 1{\cdot}5$ $\log_{10}$ units/ml), as opposed to 90% of the cultures derived from normal donors (probability of results under null-hypothesis by χ^2 analysis: $P < 0{\cdot}025$). However, the mean titre reached by responding cultures of MS patients was the same as that from normal donors.

A similar pattern of response frequencies was observed when PHA was used as an inducer. The average yields in the responding cultures was the same for both groups and reached the same value as after the Con A induction. However, only 45% of the MS patient derived leucocyte cultures produced detectable IFN, as opposed to 80% for the control cultures ($P < 0{\cdot}05$).

PBL cultures that were non-responsive to Con A were also non-producers after PHA stimulation. Forty-three cultures (23 MS patient derived and 20 controls) of those shown in Table 1 were stimulated in parallel with PHA and Con A. As can be seen in Table 2, there was a good concordance between the distributions of donors and patients whose cultures were inducible with the two mitogens. Thirteen additional patient derived PBL cultures were induced with Con A, PHA or a combination of both. Five of the 13 cultures produced IFN-γ after stimulation with Con A as well as with PHA (average yield for Con A: 2·0 $\log_{10}$ u/ml and for PHA: 2·4 $\log_{10}$ u/ml). Simultaneous stimulation of these cultures by both mitogens did not result in an increase of the IFN yield (2·0 $\log_{10}$ u/ml).

When PWM was used as an inducer, all cultures, whether from patients or normal donors, produced IFN. The same average titre was reached for both groups, the yields being approximately 10 times higher than these obtained after induction with Con A or PHA. A study of the kinetics of

Table 1. IFN production by PBL of MS patients and normal donors after *in vitro* stimulation with Con A, PHA and PWM (10 μg/ml final concentration for each mitogen)

	Stimulation with Con A			Stimulation with PWM			Stimulation with PHA		
		Responders†			Responders†			Responders†	
Donor group	Total number examined*	Number	Average titre (log_{10} u/ml)	Total number examined	Number	Average titre (log_{10} u/ml)	Total number examined	Number	Average titre (log_{10} u/ml)
MS patients	29	16 (55%)	2·2 (0·20)‡	25	25 (100%)	2·9 (0·12)	33	15 (45%)	2·2 (0·16)
Controls	20	18 (90%)	2·5 (0·16)	19	19 (100%)	3·4 (0·13)	20	16 (80%)	2·6 (0·17)
		$0{\cdot}01 < P < 0{\cdot}025$§						$0{\cdot}025 < P < 0{\cdot}05$§	

*The limited availability of leucocytes from single individuals was responsible for the varying and sometimes restricted number of cultures tested for the different mitogens and/or mitogen concentration.

†IFN titre $\geq 1{\cdot}5$ log_{10} units/ml.

‡Mean (s.e.).

§P value as obtained by χ^2 test.

Table 2. Mitogen-induced IFN in MS patient derived and control PBL cultures. Correlation between responsiveness to Con A and PHA.

		Number of cultures			
		Producing (+) or not producing (−) IFN-γ after stimulation with			
		Con A	+	−	+	−
Donor group	Total	PHA	+	−	−	+
MS patients	23		10	11	2	0
Controls	20		16	2	2	0
Total	43		26	13	4	0

production revealed that IFN-γ production continued for at least 5 days after induction with PWM, whereas for Con A and PWM it lasted only for 2 or 3 days for MS patients as well as for normal donors.

PWM is known to be mitogenic for T as well as B lymphocytes. Therefore, IFN induced by PWM might be a mixture of α, β and γ types. To investigate this possibility, the interferon obtained after PWM induction was characterized by the use of potent and specific antisera (Table 3). The IFN produced after PWM stimulation by cultures derived from both MS patients and normal blood donors was 99% or more neutralizable by anti-IFN-γ serum. There was no significant neutralization by anti-IFN-α and anti-IFN-β indicating that most if not all PWM-induced IFN was of the γ type, both in MS patients and in controls. Moreover, the PWM-induced IFN was

Table 3. Characterization of the IFN by different criteria

		Residual anti-viral activity of IFN (% of original titre) after*				
		Incubation with antisera				
Donor group	Stimulation of PBL with	Anti-IFN-α	Anti-IFN-β	Anti-IFN-γ	pH 2	56°C
MS patients	Con A	100	100	<1	<3	<3
	PHA	100	100	<10	<1	<1
	PWM (1 day)	100	100	≤1	<1	<1
	PWM (2 days)	100	100	≤1	<1	<1
	PWM (4 days)	100	100	<30‡	<3	<3
	PWM (5 days)†	100	100	<30‡		
	VSV (moi 0·1)	<18	100	ND	ND	ND
	NDV	<0·3	56	ND	ND	ND
Controls	Con A†	74	98	<10	ND	<10
	PHA†	42	68	<10	ND	<10
	PWM (1 day)	100	100	<1	≤1	≤0·3
	VSV (moi 0·1)	<3	100	ND	ND	ND
	NDV	<0·1	100	ND	ND	ND

*Unless stated otherwise, resuls were obtained on at least two individual samples.
†Results obtained on pools of four samples.
‡Decrease from 1·0 $\log_{10}$ u/ml to undetectable level.

Table 4. Influence of PWM concentrations on IFN-γ response (as expressed by IFN-γ yield and response frequency) of PBL cultures of MS patients and normal donors

	Stimulation with PWM (μg/ml)									
	1·5				5			10		
		Responders*			Responders			Responders		
Donor group	Total number examined	Number	Average titre ($\log_{10}$ u/ml)	Total number examined	Number	Average titre ($\log_{10}$ u/ml)	Total number examined	Number	Average titre ($\log_{10}$ u/ml)
MS patients	5	5	2·8 (0·15)†	4	4	3·4 (0·24)	16	16	3·0 (0·16)
Controls	7	7	3·3 (0·15)	8	8	3·4 (0·17)	11	11	3·4 (0·20)

*IFN titre $\geqslant$ 1·5 $\log_{10}$ units/ml.
†Mean (s.e.).

Table 5. Influence of Con A concentration on IFN-γ response (expressed by IFN-γ yield and response frequency) of PBL cultures of MS patients and normal donors

	Stimulation with Con A (μg/ml)											
	5			10			30			60		
		Responders†			Responders†			Responders†			Responders†	
Donor group	N_T*	Number	Average titre ($\log_{10}$ u/ml)	N_T	Number	Average titre ($\log_{10}$ u/ml)	N_T	Number	Average titre ($\log_{10}$ u/ml)	N_T	Number	Average titre ($\log_{10}$ u/ml)
MS patients	10	5 (50%)	2·3 (0·18)‡	19	9 (47%)	2·2 (0·22)	16	9 (56%)	2·2 (0·15)	6	2 (33%)	1·75 (0·19)
Controls	5	3 (60%)	2·1 (0·36)	11	10 (91%)	2·4 (0·26)	8	8 (100%)	2·6 (0·28)		Not done	
Difference in response rate		10%			44%			44%				

*N_T = Total number of cultures examined. The limited availability of leucocytes from single individuals was responsible for the varying and sometimes restricted number of cultures tested for the different concentrations used.

†IFN titre $\geq$ 1·5 $\log_{10}$ units/ml.

‡Mean (s.e.).

Table 6. IFN-α production by MS patient derived and control PBL cultures after *in vitro* stimulation with VSV, and comparison with responsiveness (IFN-γ) to stimulation with Con A

	Induction with VSV									Stimulation with Con A		
	moi = 0·1			moi = 0·5			moi = 1·0					
	Responders†			Responders†			Responders†			Responders†		
	N_T*	Number	Average titre (log_{10} u/ml)	N_T	Number	Average titre (log_{10} u/ml)	N_T	Number	Average titre (log_{10} u/ml)	N_T	Number	Average titre (log_{10} u/ml)
MS patients	22	2 (9%)	2·2 (0·25)‡	22	8 (36%)	1·9 (0·18)	31	20 (64%)	2·4 (0·16)	28	15 (53%)	2·0 (0·16)‡
Controls	15	6 (40%)	1·9 (0·24)	10	7 (70%)	1·8 (0·16)	15	13 (87%)	2·7 (0·19)	16	15 (94%)	2·2 (0·20)
Difference in response rate		31%			34%			23%			41%	
		$0·05 < P < 0·1$§			$0·1 < P < 0·25$			$0·1 < P < 0·25$			$0·01 < P < 0·025$	

*N_T = Total number of cultures examined. The limited availability of leucocytes from single individuals was responsible for the varying and sometimes restricted number of cultures tested for the different inducers.

†IFN titre ≥ 1·5 log_{10} units/ml.

‡Mean (s.e.).

§P value as obtained by χ^2 test.

neutralized by dialysis at pH 2 and incubation at 56°C. As can be seen from Table 3, Con A- and PHA-induced IFN was also completely of the γ type.

From these results one can conclude that PBL cultures of MS patients, which cannot be induced with Con A and PHA, are nevertheless able to produce IFN-γ, when stimulated by a lectin with different specificity.

Influence of mitogen concentration on the induction of IFN-γ

Threshold doses of mitogen for IFN-γ induction were determined for both cultures derived from MS patients and control donors. Con A was used at 5, 10, 30 and 60 μg/ml; and PWM at 1·5, 5 and 10 μg/ml. Even at the lowest dosage level, PWM was able to induce IFN in all cultures, whether control or MS patient derived (Table 4). Also, the yields of IFN did not differ significantly between mitogen dosage levels.

In contrast, as shown in Table 5, Con A, even when used in a dose as high as 30 or 60 μg/ml still failed to induce IFN in about half of the MS patient derived cultures, while being able to induce 90–100% of the controls. As observed in our previous studies (Vervliet *et al.*, 1983) the yields obtained in responding cultures did not differ between those derived from normal donors and MS patients. At the lowest dose, 5 μg/ml, no difference between MS patients and controls was found: in both groups the response rate was low. Only at a higher concentration of 10 μg/ml a discrimination between MS and control derived PBL became apparent; the response rate staying low for MS patients, while increasing drastically to 93% for controls.

IFN responses to NDV and VSV

In a previous study we could not detect any MS associated defectiveness in IFN response (IFN-α production) to Sendai virus. Other authors, however, have reported such defectiveness in response to various viruses, including NDV and VSV (Neighbour *et al.*, 1981; Gyodi *et al.*, 1982). In an attempt to resolve this discrepancy, experiments were done using live NDV and VSV as inducers. Cultures of the same patients or donors were stimulated with Con A as a control.

When NDV was used as an inducer virus, no difference between MS and controls was observed at a moi of 0·1 PFU/cell. NDV revealed itself as a strong inducer, giving a 100% responsiveness of the PBL cultures in nine patients and 10 controls tested, and inducing high IFN yields (3·0–3·4 $\log_{10}$ u/ml), thereby resembling Sendai virus. VSV was used at three moi (0·1, 0·5 and 1·0 PFU/cell). From the results (Table 6) one can conclude that VSV is a weak inducer of IFN: even at high doses (moi 1·0) only 87% of the PBL cultures of the controls produced IFN. This percentage still decreased at lower moi; 0·1 being the lower limit of the inducing concentrations of VSV, with only 40% of control cultures responding. At all multiplicities response frequencies of MS patient derived cultures were lower than those of control donors, the relative difference remaining the same for the three moi's tested. Statistical analysis (logit covariance analysis) showed that the dose–response

Table 7. Analysis of concordance of the IFN responsiveness of MS patient derived PBL cultures to stimulation with Con A and VSV

	Number of cultures					
		Producing (+) or not producing (−) IFN-γ after stimulation with				
	Total	Con A VSV	+ +	− −	+ −	− +	Concordancy
VSV moi 0·1	18		2	7	9	0	9/18
VSV moi 0·5	8		3	4	0	1	7/8
VSV moi 1·0	23		11	4	2	6	15/23

curves for MS patients and controls were strictly parallel, and that the displacement between the curves was highly significant ($P < 0{\cdot}02$). The average IFN yields, even for the highest dose of VSV used, were low, compared to those after induction with NDV. The same mean yields were reached for responding cultures derived from MS patients and those of controls.

Table 7 analyses the concordance of responsiveness to VSV and Con A in the MS patient group. (For the control group such an analysis was rather irrelevant since both for VSV and Con A the response rates were very high). It can be seen that the concordance differed with moi of VSV. It was good with an intermediate dose, but poor with either higher or lower doses of VSV. This can be related to the fact that, with VSV as an inducer, the response frequency increased with dose, while with Con A as an inducer it remained unaltered (see Tables 5 & 6).

DISCUSSION

We have previously reported that PBL from a significant proportion of MS patients have a defective IFN-γ response upon stimulation with Con A (Vervliet *et al.*, 1983). As a possible explanation we proposed that lymphocytes of these MS patients have a reduced reactivity to interact with mitogenic stimuli in general. This view was based on our own observations as well as on data from the literature. Indeed, similar observations using other mitogens than Con A were reported by Salonen *et al.* (1982a, 1982b). Furthermore, the defectiveness appeared not to be limited to the IFN system, since certain authors (Levy *et al.*, 1977; Dropcho *et al.*, 1982) also found evidence for reduced blastogenesis.

In the present study, done on a different population of patients, we confirmed our findings that MS is associated with a lower response rate in the *in vitro* Con A stimulation test. We also found that responses to PHA were concordant with those to Con A. In contrast, none of the MS patient derived PBL cultures were negative when PWM was used as an inducer: a 100% responsiveness of the PBL cultures was seen over a wide range of PWM doses. Also, higher IFN yields were obtained with PWM than with Con A or PHA and the IFN production after PWM stimulation continued for at least 5 days, whereas with Con A or PHA it lasted for only 2–3 days. It is known that Con A and PHA specifically stimulate T cells, but not B cells, whereas PWM activates both T and B cells (Janossy & Greaves, 1972). B cells are known producers of α or β but not γ type interferons. The possibility that the IFN produced after PWM was a mixture of α, β and γ-IFN was ruled out by characterization studies on samples obtained after 1, 2, 4 and 5 days: the IFN was shown to be of the γ type. These results clearly show that the MS associated defect is not an all-out inability of all lymphocytes to produce IFN-γ but rather a failure of responding to certain mitogenic stimuli. While Con A and PHA seem to interact with the same lymphocyte subpopulations, PWM either triggers (a) different subpopulation(s), or recognizes a broader spectrum of receptors on the membranes of T lymphocytes.

In mice concordance was found in T cell subpopulations stimulated by PHA and Con A (Archer *et al.*, 1979). In fact, this is rather concordant with our current data which show concordance in IFN-γ responsiveness of MS patients derived PBL to PHA and Con A. Further support for this view comes from the finding that simultaneous stimulation of the PBL cultures with Con A and PHA did not result in an increase of the IFN yield.

Salonen *et al.* (1982b) found significant differences between MS patients and control subjects when using various mitogens including PWM. A possible explanation for this discrepancy with our results could be that different PWM preparations differ in their biological properties (Waxdall, 1974).

From the experiments with different Con A doses the IFN-γ response appeared as an 'all or none' event: response frequencies increased with increasing doses, whereas the IFN-γ yields of responding cultures did not significantly differ between dose levels. An explanation for this could be that Con A must not only interact with the producing cells but must also trigger some accessory cell population (perhaps monocytes), which activates the producing cells. Such a mechanism could render the final yield of IFN independent of Con A dose, the critical variable being the amount or quality of accessory cells available for interaction with Con A.

Dropcho *et al.* (1982) found evidence that in MS patients there was an abnormality in an accessory cell population, because the lymphoproliferative response of unfractionated mononuclear cells from MS patients after Con A stimulation was decreased, whereas purified T cells responded normally. The authors suggested a defective interaction between accessory cells and T cells as the cause of the defective response. However, a more generalized abnormality of function of one of the accessory cell types (e.g. monocytes) was also considered possible.

Tovell *et al.* (1983) stated that in studies in which production of both viral- and mitogen-induced IFN was measured, either both or neither were found to be decreased in lymphocytes derived from MS patients. In our previous study, this was not so as we found decreased response rates to Con A, but normal response rates to Sendai virus. NDV, a classical and strong IFN-α inducer stimulated a high IFN production in 100% of the MS patient derived PBL. However, when VSV, a weak inducer of IFN-α, was used a clear and statistically significant difference in responsiveness was noted between control and MS patient derived cultures. Thus, in general, weak inducers (VSV, Con A, PHA) could discriminate between PBL cultures derived from MS patients and normal donors; strong inducers (PWM, NDV) failed to reveal such differences. The fact that, with a selected, suboptimal dose of VSV, there was concordance in response to VSA and Con A seems to suggest that a common causal factor underlies the defective responses of MS patients to those two inducers. To explain this, one should perhaps consider the role of interleukin-1 (IL-1) and -2 (IL-2) or similar lymphokines. These immunoregulatory cytokines are also involved in the regulation of IFN production. Defective production of IL-1 or IL-2 was reported in patients with systemic lupus erythemathosus (Linker-Israeli *et al.*, 1983). However, the authors were not able to trace the mechanism of this reduced production. To clarify the events underlying a defective IFN-γ response on MS patients, studies are in progress in our laboratory in which IL-1 and IL-2 production is evaluated.

We thank Dr B. Dalton (The Medical College of Pennsylvania and Hospital, Philadelphia, USA), Dr M. De Ley and Dr J. Van Damme (Rega Institute, K. U. Leuven, Belgium) for the kind gift of resp. anti-IFN-α, anti-IFN-γ and anti-IFN-β antisera; Carlo Wauters and Liesbeth Nijs for excellent technical assistance and Christiane Callebaut for typing the manuscript.

Financial support for this study was received from the Belgian Foundations for Scientific Research on Multiple Sclerosis (W.O.M.S. and B.S.M.S.) and from the A.S.L.K. Cancer Research Foundation.

REFERENCES

ARCHER, D.L., BENNETT, G.S., ULRICH, J.T. & JOHNSON, H. (1979) Immune interferon induction by T-cell mitogens involves different T-cell subpopulations. *Cell. Immunol.* **48,** 420.

BLOOM, B.R. (1980) Interferons and the immune system. *Nature*, **284,** 593.

DROPCHO, E.J., RICHMAN, D.P., ANTEL, J.P. & ARNASON, B.G.W. (1982) Defective mitogenic responses in myasthenia gravis and multiple sclerosis. *Ann. Neurol.* **11,** 456.

GYODI, E., BENCZUR, M., PALFFY, G., TALAS, M., PETRANYI, G., FÖLDES, I. & HOLLAN, S.R. (1982) Association between HLAB7, DR2 and antibody-mediated cytotoxicity without connection with the deficient interferon production in multiple sclerosis. *Hum. Immunol.* **4,** 209.

JANOSSY, G. & GREAVES, M.F. (1971) Lymphocyte activation. I. Response of T and B lymphocytes to phytomitogens. *Clin. exp. Immunol.* **9,** 483.

KURTZKE, J.F. (1970) Neurological impairment in multiple sclerosis and the disability status scale. *Acta Neurol. Scand.* **46,** 492.

LEVY, J., OPELZ, G.G., TERASKI, P.I., MYERS, L.W. & ELLISON, G.W. (1977) Histocompatibility-linked T-cell deficiency in multiple sclerosis. (abstract). *Neurology*, **27,** 372.

LINKER-ISRAELI, M., BAKKE, A.C., KITRIDOU, R.C., GENDLER, S., GILLIS, S. & HORWITZ, D.A. (1983) Defective production of interleukin 1 and interleukin 2 in patients with systemic lupus erythematosus (SLE). *J. Immunol.* **130,** 2651.

NEIGHBOUR, P.A., MILLER, A.E. & BLOOM, B. (1981) Interferon response of leukocytes in multiple sclerosis. *Neurology*, **31,** 561.

ROSE, A.S., ELLISON, G.W., MYERS, L.W. & TOURTELLOTTE, W.W. (1976) Criteria for the clinical diagnosis of multiple sclerosis. *Neurology*, **26,** 20.

SALONEN, R., ILONEN, J., REURANEN, M. & SALMI, A. (1982a) Defective production of interferon-α associated with HLA-Dw2 antigen in stable multiple sclerosis. *J. Neurol. Sci.* **55,** 197.

SALONEN, R., ILONEN, J., REUNANEN, M., NIKOSKELAINEN, J. & SALMI, A. (1982b) PPD, PWM-, and PHA-induced interferon in stable multiple sclerosis: association with HLA-DW2 antigen and clinical variables. *Ann. Neurol.* **11,** 279.

SANTOLI, D., TRINCHIERI, G. & KOPROWSKI, H. (1978) Cell-mediated cytotoxicity in humans against virus-

infected target cells. II. Interferon induction and activation of natural killer cells. *J. Immunol.* **121,** 532.

SANTOLI, D., HALL, W., KASTROKOFF, L., LISAK, L., PERUSSIA, B., TRINCHIERI, G. & KOPROWSKI, H. (1981) Cytotoxic activity and interferon production by lymphocytes from patients with multiple sclerosis. *J. Immunol.* **126,** 1274.

TOVELL, D.R., MCROBBIE, I.A., WARREN, K.G. & TYRRELL, D.L.J. (1983) Interferon production by lymphocytes from multiple sclerosis and non-MS patients. *Neurology,* **33,** 640.

VERVLIET, G., CLAEYS, H., VAN HAVER, H., CARTON, H., VERMYLEN, C., MEULEPAS, E. & BILLIAU, A. (1983) Interferon production and natural killer (NK) activity in leukocyte cultures from multiple sclerosis patients. *J. Neurol. Sci.* **60,** 137.

WAXDALL, M.J. (1974) Isolation, characterization, and biological activities of five mitogens from pokeweed. *Biochemistry,* **13,** 3671.

ZANDER, H., ABB, J., KAUDEWITZ, P. & RIETHMÜLLER, G. (1982) Natural killing activity and interferon production in multiple sclerosis. *Lancet,* **ii,** 280.