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Viral challenge in leprosy: viraemia, interferon, and specific antibody production

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Summary Following 17-D yellow fever vaccination, viraemia and specific neutralizing antibody production were assessed in groups of 12 healthy Malay controls and Malay tuberculoid and lepromatous leprosy patients. Subsequent viraemia was found in 10 healthy subjects, 9 tuberculoid patients and 8 lepromatous patients. Neither the time of appearance, chronicity, nor titre of viraemia was different amongst the three groups. Nine or 10 individuals from each of the 3 subject groups developed specific neutralizing antibody. Prior to vaccination, the ability of peripheral blood leucocytes to produce interferon *in vitro* after stimulation with Newcastle Disease Virus, was studied. Leucocytes from all the healthy subjects and patients produced significant amounts of interferon. Neither lepromatous nor tuberculoid patients' leucocytes produced levels of interferon different from healthy controls. A tendency was observed for lepromatous patients to produce decreased amounts of interferon *in vitro* as compared to tuberculoid patients ($P=0{\cdot}06$).

Introduction

Clinicians generally agree, and a number of studies have supported the view, that leprosy patients appear to handle other infections normally[1] and are not predisposed to immunologically mediated disease or malignancy.[2–4] As a prerequisite for leprosy vaccines to prove effective, individuals at risk in endemic countries should be capable of responding in a normal fashion to unrelated infectious agents. In order to experimentally test this issue, we performed a study in Malaysia utilizing well classified lepromatous and tuberculoid patients and healthy controls, which assessed *in vitro* leucocyte interferon production and *in*

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vivo viraemia and specific neutralizing antibody development, following the challenge of subjects with a non-endemic virus, live attenuated yellow fever.

Materials and methods

SUBJECTS

Subjects included 12 healthy Malay staff members of the National Leprosy Control Center, Sungei Buloh, Malaysia, 12 Malay lepromatous leprosy patients, and 12 Malay tuberculoid leprosy patients. None of the subjects had a prior history of yellow fever vaccination, encephalitis, dengue or travel outside Malaysia. Disease classification was based on clinical and histologic findings (Ridley, D.S.) and lepromin skin testing. Subjects were vaccinated subcutaneously with 0·5 cc of a 17-D vaccine strain of yellow fever. Viraemia was assessed prior to vaccination and 4 or 5 days, and in some instances 6 days later, and circulating neutralizing antibody were determined and quantitated prior to vaccination and 4, 5 and 12 days later. Prior to vaccination, the ability of peripheral blood leucocytes to produce interferon *in vitro* was assessed.

VIRAEMIA

Viraemia following yellow fever vaccination was assayed in pig kidney (PS) cells grown in Leibovitz L15 medium with 3% inactivated foetal calf serum following the method of Madrid and Porterfield[5] with minor modifications. Undiluted human serum was added to a suspension of 3×10^5 PS cells/ml, using 0·1% ml serum to 2·5 ml cells in one 50 mm plastic petri dish or 0·05 ml serum to 0·5 ml cells in each of two wells in disposable FB-16-24 plastic containers, and cultures were overlaid with an equal volume of carboxymethyl cellulose overlay 2 h later. After 5 (or occasionally 7) days incubation at 35°C, preparations were rinsed with normal saline, stained with naphthalene black, and any plaques visible were counted.

YELLOW FEVER ANTIBODY

Neutralization tests were performed in disposable plastic trays. One volume (0·2 ml) of an appropriate dilution of yellow fever vaccine suspension was mixed with one volume of heat inactivated (56°C/30 min) human serum diluted, 1:10, 1:40, 1:160 and 1:640, and the serum virus mixtures were held in the wells of plastic plates overnight at 4°C. Two volumes of PS cells (0·4 ml of 3×10^5/ml) were then added, and the mixtures incubated at 35°C for 2 hs after which 0·4 ml of carboxymethyl cellulose overlay was added to each well, and the preparations incubated at 35°C for 5–7 days. Confluent or semi-confluent plaques developed in

the absence of yellow-fever neutralizing antibodies; sera which reduced the plaque count to below 50% of that produced in control wells were recorded as positive.

INTERFERON PRODUCTION *IN VITRO*

From each subject 20–25 ml of venous blood with phenol-free heparin (30 iu/ml) were placed in a vessel to which 6% dextran (4 to 5 ml) was added. Ten millilitre portions in sterile screw-capped tubes were inclined at 45° and incubated at 37°C for 40 min. The plasma was then removed, pooled, and centrifuged at 1000 rpm for 10 min at room temperature. The leucocyte containing sediment was suspended in Eagles media plus 10% foetal calf serum. Cell counts were adjusted to 3×10^6 cells/ml in screw-capped tubes and inocculated with 0·2 ml of undiluted allantoic fluid containing Newcastle Disease Virus (NDV1) with viral hemagglutinin titres of 1/640 or greater. After 24-h incubation in a roller drum at 37°C the fluid was harvested for interferon assay.

Interferon was assayed on fluid dilution by assessment of inhibition of rhinovirus cytopathic effects on WI38 human diploid lung cells utilizing the method of Wheelock.[6] In each test a standard interferon preparation was incorporated.

Results

VIRAEMIA

The results are presented in Table 1. Some early sera were toxic to the cell cultures; consequently it was impossible to detect viraemia in these samples. As expected, viraemia was never present on day 0, but was present in 6 samples on day 4, in 26 samples on day 5, and in 15 samples on day 6. Viraemia was detected at least once in 10/12 normal subjects, in 8/12 patients with lepromatous leprosy and in 9/12 patients with tuberculoid leprosy. There were no significant differences between these three groups in the time of appearance, duration or magnitude of the viraemia.

YELLOW FEVER ANTIBODY

The results are presented in Table 1. Neutralizing antibody titres of 10 or less were regarded as negative, and titres of 20 and above as positive. By these criteria, 9 of the pre-vaccination samples (day 0) appeared to have neutralizing antibodies against yellow fever virus. Since none of the subjects had received prior yellow fever vaccine, and since yellow fever does not occur in nature in Asia, these positive findings presumably reflect cross-protection produced by antibodies

Table 1.

Clinicopathologic classification	Mitsuda ± (induration)	Yellow Fever viraemia* days				Yellow Fever antibody days				Newcastle Disease Virus induction of leucocyte interferon (reciprocal of plaque formation)
		0	4	5	6	0	4	5	12	
All L										
(#12) L	−(5)	?	?	20	10	10	10	< 10	20	10^{-3}
(#14) L	−(0)	0	0	0	0	< 10	< 10	10	80	$10^{-2.5}$
(#15) BL	−(5)	0	SC	5	20	10	10	10	80	$10^{-1.85}$
(#17) L	+(9)	0	0	0	0	< 10	< 10	10	80	$10^{-2.25}$
(#18) BL	−(5)	?	?	10	15	10	< 10	< 10	80	10^{-3}
(#27) BB/BL	−(0)	0	0	0		< 10	< 10	< 10	20	$10^{-2.1}$
(#28) L	−(5)	0	Conf	10		< 10	< 10	< 10	40	$10^{-2.27}$
(#29) BL	+(8)	0	0	3		20	20	20	40	$10^{-2.9}$
(#31) BL	−(0)	0	0	4		20	20	20	80	$10^{-3.25}$
(#32) L	−(2)	?	?	15		20	20	20	20	$10^{-2.7}$
(#33) L	lacking	?	?	15		20	20	NT	20	$10^{-2.3}$
(#35) L	−(0)	?	?	0		10	10	10	40	$10^{-2.5}$

All T										
(#1) BT	−(3)			20	5	NT	NT	< 10	10	10^{-3}
(#9) T	lacking			4	3	NT	NT	< 10	20	$10^{-2\cdot}$
(#10) T	+ (14)			0	0	NT	NT	10	40	$10^{-2\cdot8}$
(#11) BT	+(10)	0	SC	12	10	20	< 10	10	20	$10^{-3\cdot5}$
(#13) T	+(8)	?	?	3	8	10	10	10	20	$10^{-2\cdot7}$
(#16) T	lacking	0	Conf	10	10	20	20	20	20	10^{-3}
(#19) BT	−(4)	0	0	0	0	< 10	< 10	< 10	80	$10^{-3\cdot15}$
(#21) T	+(10)	0	SC	0		< 10	< 10	< 10	80	$10^{-2\cdot1}$
(#23) T	+(14)	?	?	20		20	20	20	40	$10^{-2\cdot}$
(#25) T	+(12)	0	0	10		< 10	< 10	< 10	20	$10^{-2\cdot7}$
(#26) BT	+(9)	0	0	3		< 10	< 10	< 10	80	$10^{-2\cdot75}$
(#34) T	+(11)	0	0	0		10	10	10	20	$10^{-4\cdot3}$
All normal										
(#2)	+(10)	0	0	10	2	< 10	< 10	< 10	40	$10^{-2\cdot}$
(#3)	+(18)	?	?	8	10	< 10	< 10	< 10	10	$10^{-2\cdot4}$
(#4)	+(7)	?	?	20	20	10	10	10	40	$10^{-2\cdot}$
(#5)	+(9)			Conf	2	NT	NT	10	40	$10^{-3\cdot3}$
(#6)	+(9)	0	0	SC	SC	10	< 10	< 10	10	$10^{-3\cdot15}$
(#7)	+(10)	0	0	5	3	< 10	< 10	< 10	40	$10^{-3\cdot5}$
(#8)	+(20)			Conf	Conf	NT	NT	< 10	20	$10^{-2\cdot35}$
(#20)	+(12)	?	?	0		< 10	10	10	20	$10^{-2\cdot}$
(#22)	+(10)	0	Conf	5		20	20	> 20	80	$10^{-2\cdot2}$
(#24)	+(9)	0	0	0		< 10	< 10	< 10	80	$10^{-2\cdot}$
(#30)	+(10)	0	0	8		20	20	20	40	10^{-3}
(#36)	lacking	?	?	1		10	< 10	10	40	$10^{-2\cdot3}$

?, toxic; SC, semi-confluent; Conf, confluent; *, plaques per 0·1 ml serum; NT, not tested.

against antigenically related flaviviruses which do occur in Malaysia, of which dengue and Japanese encephalitis viruses are the two most probable candidates. The day 4 and day 5 samples gave results virtually indistinguishable from those obtained with the day 0 samples, but by day 12 only 3 sera remained antibody negative, and these came from subjects who were viraemic on days 5 and 6. Antibody titres tended to be higher in the lepromatous leprosy patients than in the other groups. All the subjects who failed to produce a detectable viraemia nevertheless responded with the production of yellow fever antibodies.

INTERFERON PRODUCTION *IN VITRO*

All of the subjects studied produced significant amounts of interferon *in vitro* following stimulation of peripheral blood leucocyte cultures. For normal subjects interferon production averaged $10^{-2\cdot73\pm0\cdot27(\text{S.D.})}$, and correspondingly tuberculoid patients $10^{-2\cdot95\pm0\cdot30(\text{S.D.})}$, and lepromatous patients $10^{-2\cdot55\pm0\cdot18(\text{S.D.})}$. These differences in interferon production between the three groups of patients were not significant. However, lepromatous subjects' interferon production was less than tuberculoid patients' ($P=0\cdot06$).

Discussion

Wheelock *et al.*[7] demonstrated previously that viraemia could be identified in 10 of 15 normal subjects following vaccination with the 17-D strain of yellow fever virus, and by 10 days following vaccination, yellow fever antibody was detectable in all the subjects. These current studies are in essential accord with those findings. In our studies the titre and period of viraemia, following viral challenge *in vivo* and the production of specific neutralizing antibody appears to be normally generated in both lepromatous and tuberculoid patients. Also, these studies demonstrate that production of interferon to viral challenge by peripheral blood mononuclear cells *in vitro* in leprosy patients across the spectrum, does not appear aberrant. Control of viral infection involves a complex network which includes antibody, cellular immunity, and interferon which act in an integrated manner. These studies support that in leprosy patients this network is generally intact.

Though patients with lepromatous leprosy are known to produce a polyclonal hyperglobulinemia[8–11] that can result in a wide variety of falsely positive serologic tests,[12–16] antibody production in leprosy is not, however, conceived to be generally aberrant. In fact lepromatous patients were found to produce higher titres of agglutinins to H-antigens, in response to typhoid vaccine, than healthy military recruits.[17] Indeed, antibody to a *Mycobacterium leprae*-specific phenolic glycolipid is regularly produced in lepromatous leprosy and in high titre.[18] Our studies further support the concept that antibody to specific pathogens is not abnormally generated in leprosy patients.

Glasgow and Bullock[19] previously demonstrated that mice heavily infected with *M. lepraemurium* following intraperitoneal challenge with chikungunya virus produced distinctly lower levels of interferon than control mice, uninfected with *M. lepraemurium.* Mixed peritoneal cells from infected mice also reflected these *in vivo* findings and demonstrated decreased interferon production to chikungunya virus. It has been postulated that human lepromatous leprosy might also be associated with a defect of interferon producing capacity or other responses to viral challenges. In this respect a number of clinical reports have indeed suggested that lepromatous leprosy patients are more susceptible to variola[20] and demonstrate slow resolution of inoculation lesions and vaccinia gangrenosa following vaccination,[21,22] an indication of impaired cellular immunity. The present study found leprosy patients had no observed aberrancy in protective immunity to yellow fever. The decreased production of interferon *in vitro* by lepromatous as compared to tuberculoid patients ($P = 0{\cdot}06$) found in these studies is, however, of some interest.

Though interferon production has been classically considered to be induced by viruses, parasites,[23–26] and bacterial products, including endotoxin[27–30] tuberculin,[31] and poly RI–poly IC[32–35] are potent interferon inducers. Interferon has also been shown to inhibit *in vivo* multiplication of various intra- and extracellular pathogens other than viruses including pneumococci,[36] listeria,[37] trachoma,[38] cryptococcus,[19] *P. berghei*,[39] and certain tumours not known to be caused by viruses.[40] Levy *et al.*[41] found that the potent interferon inducers poly IC and Tilerone inhibited mouse footpad multiplication of *M. leprae.* Nogueira *et al.*[42] found peripheral blood mononuclear cells from lepromatous leprosy patients, even those on long-term therapy, deficient in their capacity to release gamma interferon *in vivo* in response to both mitogen and *M. leprae.* In those studies interleukin 2 was found to restore the decreased gamma interferon production of lepromatous leprosy in response to specific antigen or mitogen. Gamma interferon is evoked by specific cellular memory and these studies do not clarify if this fraction, especially that induced by *M. leprae* and potentially protective, is deficient.

There is considerable evidence on both sides of the issue of whether the immune defect in lepromatous leprosy is due to a specific defect[43–46] in host defence mechanisms against *M. leprae* or secondary to some more generalized anergy.[47–51] There has also been conjecture that certain individuals are genetically incapable of mounting an appropriate protective response thereby being predisposed to the development of leprosy, or alternatively, that the development of leprosy is due entirely to environmental exposure to the bacillus, wherein possibly the timing, route, and degree of exposure are crucial factors in determining whether overt disease does or does not become manifest. Certainly these two propositions need not be mutually exclusive and most likely both heredity and environmental factors interplay to determine the outcome in

individuals exposed to *M. leprae*. In any event these current studies lend support to the concept that leprosy patients do not present a generalized immune defect.

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