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Natural killer cell activity during measles

D. E. GRIFFIN*†, B. J. WARD*, E. JAUREGUI‡, R. T. JOHNSON† & A. VAISBERG§
*Departments of *Medicine and †Neurology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA, ‡National Institute of Child Health, Lima, and §Department of Microbiology, Universidad Peruana Cayetano Heredia, Lima, Peru*

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SUMMARY

Natural killer cells are postulated to play an important role in host anti-viral defences. We measured natural killer cell activity in 30 individuals with acute measles (73 ± 21 lytic units (LU)/10^7 cells) and 16 individuals with other infectious diseases (149 ± 95 LU) and found it reduced compared with values for adults (375 ± 70 LU; $P < 0.001$) or children (300 ± 73 LU, $P < 0.01$) without infection. Reduced natural killer cell activity was found in measles patients with (84 ± 30 LU) and without (55 ± 18 LU) complications and was present for at least 3 weeks after the onset of the rash. Activity was increased by *in vitro* exposure of cells to interleukin-2. Depressed natural killer cell activity parallels in time the suppression of other parameters of cell-mediated immunity that occurs during measles.

Keywords natural killer cells measles interferon interleukin-2

INTRODUCTION

Natural killer (NK) cells have the ability to lyse virus-infected cells, as well as tumour cells (Herberman *et al.*, 1979; Rager-Zisman & Bloom, 1982) and are important in host resistance to viral infections in some experimental systems (Biron & Welsh, 1982; Bukowski *et al.*, 1983). Since NK activity is increased by exposure of cells to interferon (IFN) (Trinchieri & Santoli, 1978) it is reasonable to assume that the IFN induced by viral replication *in vivo* will serve not only to induce an anti-viral state in nearby cells, but also to induce NK activity in circulating leucocytes. However, in natural viral infections the relationship between viral replication and host responses is complex. Many factors influence the functional state of various cell populations and viral infection often alters expected leucocyte responses (Sissons & Oldstone, 1980). Therefore, study of natural disease is necessary for a full understanding of these interactions.

Measles has long been recognized as a disease associated with a variety of abnormal cellular immune responses along with vigorous anti-viral host defences. Immunological abnormalities can be demonstrated both *in vivo* by diminished delayed-type hypersensitivity skin test responses (von Pirquet, 1908; Tamashiro, Perez & Griffin, 1987) and *in vitro* by diminished lymphoproliferative responses (Finkel & Dent, 1973; Hirsch *et al.*, 1984). Most deaths during measles are due to secondary bacterial and viral infections (Morley, 1969; Beckford, Kaschula & Stephen, 1985). Activity of potentially important cytotoxic cells such as cytotoxic T lymphocytes, K cells and NK cells, has had only limited assessment during acute measles (Yamanaka, Chiba & Nakao, 1976; Kreth & Wiegard, 1977; Kreth, ter Meulen & Echert, 1979; Sissons *et al.*, 1985). However, based on the results of *in vitro* infection of peripheral blood mononuclear cells (PBMC) with a vaccine strain of measles virus, it has been postulated that NK cell function is impaired (Casali *et al.*, 1984). In order to determine the effect of acute natural measles on NK activity we have studied children at various times during the disease and have correlated NK activity with other parameters of cell-mediated immunity. We show that NK activity is reduced in measles and can be partially restored by culture with interleukin-2 (IL-2). This reduction occurs at the same time, but does not correlate directly with other parameters of immune dysfunction.

Correspondence Diane E. Griffin, MD, PhD, Department of Neurology, Meyer 6-181, The Johns Hopkins Hospital, Baltimore, MD 21205, USA.

PATIENTS AND METHODS

Patients

Patients with acute (within 3 weeks of the onset of rash) natural measles virus infections (31 samples from 30 patients aged 5 months to 10 years; mean 3·0 years) seen in outpatient and inpatient health care facilities in Lima, Peru, were studied. Nine had uncomplicated measles and 21 had measles complicated by pneumonia or diarrhoea. Peruvian adults without evidence of infection (13 samples from 12 individuals), children with other infectious diseases (16 children; 10 with varicella; two with pertussis; one with rubella; one with rash; and two with polio; mean age 4·8 years, range 15 months - 10 years) and 15 healthy

children followed as outpatients for seizure disorders (mean age 8·2 years, range 18 months – 16 years) served as controls. All children (those with seizures, measles and other infections) were drawn from the same general population in Lima. Nutritional status was not formally assessed but is generally good in this largely Quechua population and did not vary between the groups.

Interferon

IFN was assayed by incubating serum, diluted two-fold beginning at 1/8 or 1/10, overnight on WISH cells (American Type Culture Collection, Rockville, MD) grown in 96-well microtitre plates. Cells were then challenged with 100 tissue culture 50% infectious doses of vesicular stomatitis virus (Griffin & Hess, 1986). Cell viability was evaluated at 48 h by staining with crystal violet. The IFN titre was the highest dilution of serum which protected the cells 50%.

NK assays

Blood was collected, heparinized (20 U/ml blood) and the PBMC separated on Ficoll/Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) gradients. Cells were washed with HBSS and resuspended in RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 5% fetal bovine serum (FBS; GIBCO), 10 mM HEPES, pH, 7·4 and gentamicin (50 μg/ml). K562 (erythroid leukaemia) cells (Klein *et al.*, 1976) were used as target cells (West *et al.*, 1977) and were grown in RPMI/5% FBS, passed the day before use, and labelled with ^{51}Cr (New England Nuclear, Boston, MA). The assay was conducted in round-bottomed microtitre plates as previously described (Griffin & Hess, 1986) using 5×10^3 target cells/well and PBMC effector-to-target cell ratios (E:T) of 30:1, 10:1, 3:1, and 1:1. PBMC from controls and individuals with measles were assayed simultaneously. Assays were performed in triplicate in a volume of 200 μl/well. Chromium release was measured after 4 h at 37°C. Specific lysis was calculated using 1% SDS to determine maximum lysis. NK cell activity was expressed as lytic units (LU)/10^7 PBMC as determined by least squares analysis. One LU was defined as the number of effector cells required for 20% specific lysis of 5×10^3 target cells.

In vitro *activation of cytotoxic cells*

PBMC in RPMI/5% FBS were cultured overnight at a concentration of 10^6 cells/ml in the presence of 100 U/ml of recombinant IL-2 (Biogen) and then assayed for lytic activity against ^{51}Cr-labelled K562 cells as described above.

Lymphoproliferation

PBMC at a concentration of 10^6 cells/ml in RPMI/5% FBS were stimulated with 2·5 μg/ml phytohaemagglutinin (PHA; Pharmacia) for 48 h, pulsed overnight with 1 μCi ^3H-thymidine (^3H-TdR)/well to determine PHA-induced proliferation. Fresh PBMC were cultured for 24 h with ^3H-TdR to determine spontaneous proliferation. Cells were harvested on glass fibre filters and ^3H-Tdr incorporation was determined as previously described (Hirsch *et al.*, 1984).

Soluble IL-2 receptor and CD8 assays

Soluble IL-2 receptor and soluble CD8 were measured in plasma by enzyme immunoassays using reagents and according to directions supplied by the manufacturer (T Cell Sciences, Cambridge, MA) (Griffin *et al.*, 1989). Data are expressed in U/ml based on standards supplied by the manufacturer.

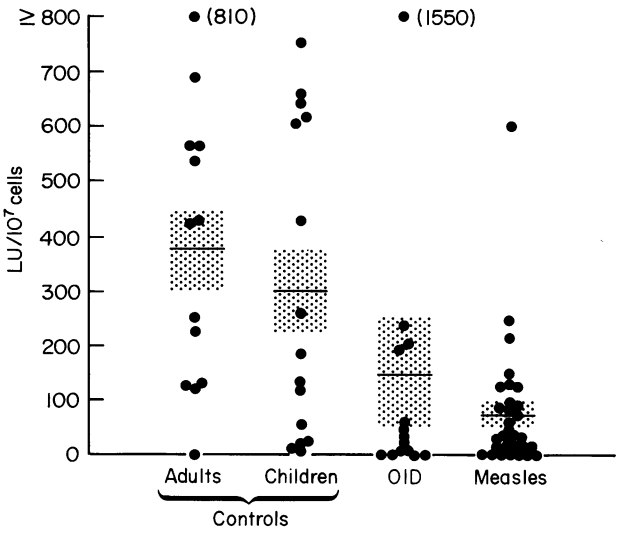

Fig. 1. Natural killer (NK) activity in lytic units (LU)/10^7 cells of PBMC from individuals with measles, other infectious diseases (OID) and controls. NK activity was lower in measles than in either adults ($P < 0.0001$) or children ($P < 0.01$) without infection. NK activity in OID controls was not significantly higher than in measles, but like measles was lower than in either adults ($P < 0.001$) or children ($P < 0.05$) without infection.

Statistical analysis

Data were analysed using the non-parametric Mann–Whitney *U*-test on Statview 512 software (Brainpower, Calabasas, CA).

RESULTS

NK activity for measles patients was compared with three different control groups derived from the Peruvian population: children with other infectious diseases; healthy children on seizure control medication; and adults without evidence of infection (Fig. 1). Measles patients as a group had lower NK activity (73 ± 21 LU) than each of the control groups and this was statistically significant in comparison to children (300 ± 73 LU, $P < 0.01$) and to adults (375 ± 70 LU, $P < 0.0001$), without evident infection suggesting that NK activity is decreased during measles. NK activity in measles was not significantly different from NK activity during other infections (149 ± 95 LU, $P > 0.5$) which were also significantly lower than levels in control children ($P < 0.05$) and adults ($P < 0.001$). Suppression of NK activity was profound in many individuals. PBMC from 11 of 31 measles patients had < 10 LU of NK activity while PBMC from only two out of 28 control samples from uninfected individuals had this little activity ($\chi^2 = 6.8$, $P < 0.01$) (Fig. 1).

Age has little effect on NK activity in healthy individuals (Pross & Baines, 1982) and low levels of NK activity could not be attributed to age differences between children with measles (mean age 3·0 years) and control children (mean age 8·2 years). The youngest control children ($n = 5$; mean age 3·3 years) had NK activity (205 ± 117 LU) similar to the entire group of control children (300 ± 73 LU, $P = 0.46$) and measles patients over the age of 3 years ($n = 11$) had similar NK activity (57 ± 22 LU) to those aged 3 years or less ($n = 20$, 82 ± 31 LU, $P = 0.28$).

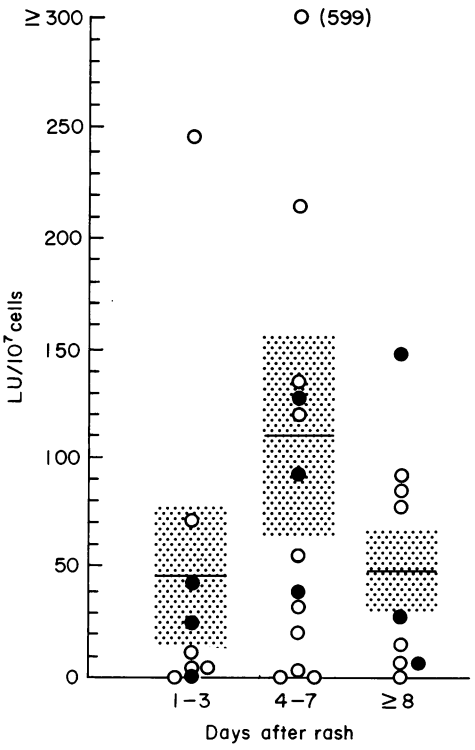

Fig. 2. Natural killer (NK) activity in lytic units/10^7 cells of PBMC from individuals with measles with (O) and without (●) complications at various times after the onset of the rash. Levels of NK activity were low at all times examined.

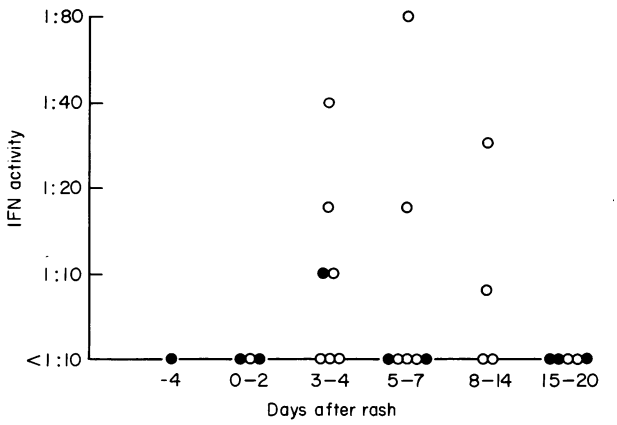

Fig. 3. Interferon (IFN) activity in the plasma of individuals with measles with (O) and without (●) complications at various times after the onset of the rash.

Low levels of NK activity occurred at all times after the onset of rash without evident differences over the 3-week time period examined (Fig. 2). One patient studied prior to the onset of the rash had NK activity of 25 LU; however, no other individuals were available from this early stage in the disease to determine whether NK activity might be more normal or elevated before the onset of the rash. One patient studied at two times had similar values of 31 LU on day 6 and 5 LU on day 10 after onset of rash. There were no patients available for study at >3 weeks after onset of rash to determine when NK activity returned to normal.

Low NK levels occurred in patients with uncomplicated measles as well as in those with complications (Fig. 2). There was no difference between the average NK activity in patients with complications of pneumonia or diarrhoea (84±30 LU), usually due to secondary infections, and patients with uncomplicated measles (55±18 LU).

Very little IFN activity was detected in plasma from these patients (Fig. 3). IFN was present in only eight of 27 plasma samples from measles patients tested. The samples with IFN were all taken during the period of 3–14 days after the onset of the rash and most were from children hospitalized with pneumonia or diarrhoea. These data suggest the possibility that plasma IFN at this stage of infection was induced by secondary infection rather than by infection with measles virus. Patients with measurable IFN activity in plasma did not have significantly higher levels of NK activity ($n=8$, 119±70 LU) than those without measurable plasma IFN ($n=19$, 66±17 LU, $P>0.5$). IFN was not measurable in the plasma of 13 out of 14 children with seizures or in seven out of eight children with other infectious diseases.

To determine whether low NK activity correlated with other parameters of immune suppression PHA-induced proliferation of PBMC was measured (Table 1). No correlation was noted between mitogen-induced proliferation and NK activity. Proliferative responses in those with <10 LU NK activity were not significantly different ($P>0.1$) from the proliferative responses in those with ≥10 LU NK activity. In measles, immune

Table 1. Correlation of natural killer (NK) cell activity with other parameters of immune function and activation

	NK activity					Correlation	
Parameter	<10 LU	(*n*)	>10 LU	(*n*)	*P*	*r*	*P*
Cell proliferation (ct/min)							
PHA-induced	49 913±8289	(8)	61 514±6380	(19)	>0·4	0·20	>0·1
Spontaneous	4903±1437	(8)	7995±1789	(13)	>0·2	0·27	>0·1
Soluble T cell antigens (u/ml)							
IL-2 receptor	4201±1279	(9)	4121±627	(14)	>0·5	0·014	>0·1
CD8	9461±1350	(10)	5344±1510	(14)	>0·5	0·07	>0·1

LU, lytic unit; PHA, phytohaemagglutinin; IL-2, interleukin-2.

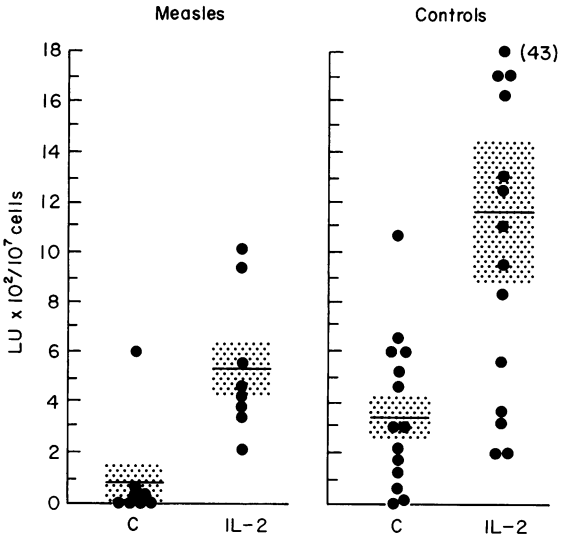

Fig. 4. NK activity in lytic units/10^7 cells of PBMC from measles and control children cultured overnight without additives (C), or with 100 U recombinant interleukin-2 (IL-2).

suppression occurs in the context of immune activation as part of a vigorous response to the infecting agent (Griffin *et al.*, 1986, 1989). In order to determine whether NK activity correlated with parameters of immune activation, NK activity was compared with spontaneous proliferation of PBMC and plasma levels of soluble IL-2 receptor and soluble CD8, all elevated during measles (Table 1) (Griffin *et al.*, 1989). No correlations were identified and no significant differences were present in the data from the group with < 10 LU NK activity compared with the group with > 10 LU.

To determine whether the NK cells from measles patients could respond to *in vitro* activation, PBMC from eight measles patients and 12 control children were studied for activation of NK activity by *in vitro* culture with exogenous recombinant IL-2 (Fig. 4). Lytic activity increased in both measles patients ($P < 0{\cdot}02$) and controls ($P < 0{\cdot}001$) after culture with IL-2.

DISCUSSION

NK cell activity is decreased below normal values during measles rather than, as might have been predicted elevated. Values were low at all times examined, until 3 weeks after the appearance of the rash. The levels of NK activity in measles did not correlate with spontaneous or mitogen-induced proliferation of PBMC or plasma levels of soluble IL-2 receptor or soluble CD8. Lytic activity was increased in response to IL-2. These data show that NK activity is generally reduced in measles but responsive to the effects of IL-2.

The reason for reduced NK activity in measles is not clear. Culturing measles virus with normal PBMC *in vitro* decreases NK activity possibly due to viral replication in and functional impairment of the cells responsible for cell lysis (Casali, Rice & Oldstone, 1984). The relevance of this *in vitro* observation for natural infection is not clear. During natural infection no virus is recoverable from PBMC a few days after the appearance of the rash (Gresser & Chany, 1963; Whittle *et al.*, 1978). It is possible that previous viral replication in PBMC prior to the onset of the rash impairs activity of the NK cell population. NK cells are often present at sites of virus infection in experimental systems (Griffin & Hess, 1986; Natuk & Welsh, 1987) and may therefore be sequestered in such sites as the lung, liver, skin and lymphoid tissue during measles and thus be absent in blood. However, PBMC can be activated to near normal levels of NK activity by IL-2 *in vitro* suggesting that a cell population with NK potential is present in the peripheral blood during measles.

NK activity has been studied during several other acute and chronic human virus infections. After administration of the 17 D vaccine strain of yellow fever virus, NK activity increases in coincidence with spontaneous proliferation of PBMC and then returns to normal (Fargreus *et al.*, 1982). During cytomegalovirus-induced mononucleosis NK cytotoxicity is unchanged (Rinaldo *et al.*, 1983) while it is depressed in Ross River virus-induced polyarthritis (Aaskov, Fraser & Dalgliesh, 1981), in condyloma acuminata (Cauda *et al.*, 1987) and in HIV infection (Bonavida, Katz & Gottlieb, 1986; Fontana *et al.*, 1986; Katzman & Lederman, 1986). Our data indicate (Fig. 1) that NK activity is decreased in several other infectious, mostly viral diseases. These studies suggest that decreased NK activity, rather than increased activity is more common in human viral infections.

We were not able to detect circulating IFN in most of these patients. Similar data have been obtained in measles using a radioimmunoassay (Shiozawa *et al.*, 1988) rather than a bioassay. In other acute natural and experimental viral infections circulating IFN is detectable primarily during the phase of active virus replication before the appearance of the specific immune response, and not at later times (Wheelock & Sibley, 1965; Luby *et al.*, 1969; Aaskov *et al.*, 1981; Levis *et al.*, 1984; Burke & Morrill, 1987; Tilles, Balkwill & Davilla, 1987). In general, clinical symptoms occur relatively late in the infectious process. In measles the incubation period of 10–14 days represents the period of viral replication. The rash marks the onset of the specific immune response and the beginning of immune-mediated virus clearance (Graves *et al.*, 1984; Moench *et al.*, 1988). Measles virus can induce IFN *in vitro* (Volckaert-Vervliet & Billiau, 1977) and increased serum IFN is found 8–11 days after measles immunization (Petralli, Merigan & Wilbur, 1965). It is therefore likely that plasma IFN levels are elevated at earlier times during the incubation period. The half-life of IFN in blood lasts only hours but the biological effects last for days (Witter *et al.*, 1987). Serum levels of IFN in many other natural infections have proved to be low, although anti-viral effects of IFN were evident (Ferbus *et al.*, 1988). It is possible that NK activity is elevated prior to the onset of the rash although the single patient studied early did not have detectable IFN and had low NK activity.

Reduced NK activity has been described as a consequence of various immunosuppressive regimens (Waltzer *et al.*, 1985; Muller *et al.*, 1987), after thermal injury (Klimpel *et al.*, 1986), in chronic-fatigue syndrome (Kibler *et al.*, 1985; Caligiuri *et al.*, 1987), systemic lupus erythematosus (Katz *et al.*, 1982), multiple sclerosis (Benczur *et al.*, 1980; Hirsch & Johnson, 1985) and advanced malignancy (Steinhauer *et al.*, 1982). Certain viral proteins appear to be able to alter NK activity when added to PBMC *in vitro* (Casali *et al.*, 1981; Harris *et al.*, 1987). Although a retroviral protein decreases cytotoxicity (Casali *et al.*, 1981), measles virus glycoproteins are reported to increase cytotoxicity *in vitro* (Harris *et al.*, 1987). Animal models of stress have shown

decreased NK activity mediated by endogenous opioid peptides released in response to stress (Shavit *et al.*, 1984). The frequency with which depressed levels are found in natural viral infections would suggest the importance of a broadly applicable mechanism such as stress (Shavit *et al.*, 1984), fever (Dinarello *et al.*, 1986), or presence of an immunologically induced circulating factor such as prostaglandin (Lang *et al.*, 1982) or transforming growth factor-beta (Rook *et al.*, 1986) as a cause for the suppression.

In measles and other viral infections there is considerable evidence of immune system activation coincident with a lack of responsiveness to mitogens and skin test antigens and it is likely that the two seemingly disparate observations are linked (Griffin *et al.*, 1989). The observation that IL-2 is able to restore partially NK activity suggests integrity of at least a subset of lymphokine-activatable cells. IL-2 also partially restores the NK activity of PBMC from AIDS patients (Bonavida *et al.*, 1986). There is considerable evidence that even baseline NK activity is dependent on IL-2 stimulation and that supplemental IL-2 further increases the NK activity (Domzig, Stadler & Herberman, 1983). Lymphokine production may be deficient during measles (Joffe & Rabson, 1981) and there is some evidence that limited production of IL-2 *in vitro* contributes to suppressed mitogen-induced proliferation (Griffin *et al.*, 1987). It is possible that low NK activity is another manifestation of a relative deficiency in IL-2 production.

Abrogation of NK activity in mice increases susceptibility to some (Shellam *et al.*, 1981; Bukowski *et al.*, 1983; Godney & Gauntt, 1986), but not all (Hirsch, 1981) virus infections. Human NK cells have also been demonstrated to have antibacterial activity (Garcia-Penarrubia *et al.*, 1989). It is possible therefore that this defect in NK activity contributes, along with abnormalities of lymphocyte (von Pirquet, 1908; Finkel & Dent, 1973; Hirsch *et al.*,1984; Tamashiro *et al.*, 1987) and monocyte (Griffin *et al.*, 1987) function, to the overall pattern of increased susceptibility to secondary infections which occurs during measles.

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