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Characteristics of asymptomatic secondary immune responses to measles virus in late convalescent donors

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SUMMARY

Among 44 fully protected, late convalescent adults re-exposed to measles, four developed an asymptomatic secondary immune response (SIR) with a significant increase in measles virus (MV)-specific IgG and low IgM. The boosted antibodies were mainly of the IgG1 subclass and reacted with the nucleoprotein and the haemagglutinin protein. About 30 weeks after re-exposure, antibody levels had decreased by 35–50%, suggesting that the booster effect may only be transient. SIR was only found in individuals with a pre-exposure IgG level below a well defined threshold. Antibody levels above this threshold fully protected against SIR. SIR seems to be an 'all or none response' where the magnitude of increase in specific IgG is independent of pre-exposure antibody levels as long as these are below the above threshold. In combination with pre-exposure neutralizing and haemagglutination inhibiting titres, a threshold was defined below which SIR is likely to occur. This may be useful to predict susceptibility to SIR in a given population, since individuals undergoing clinically inapparent SIR are among seropositive subjects, the most likely candidates to support transmission of virus.

Keywords measles secondary immune response immunoglobulin subclasses

INTRODUCTION

Measles infection and successful vaccination normally result in life-long protective immunity [1]. After the introduction of vaccination, measles morbidity and mortality dramatically decreased [2], but despite high vaccination coverage measles outbreaks occur [3,4], because the virus continues to circulate in seronegative individuals. In populations with vaccination rates exceeding 99%, outbreaks were observed in individuals with primary or secondary vaccine failure [2,4]. However, there is also evidence that measles virus (MV) can circulate in seropositive populations [4,5]. The characteristics of this transmission are so far only poorly understood. It is reasonable to assume that people susceptible to develop a secondary immune response (SIR) after re-exposure to measles are the most likely seropositive candidates to support viral transmission. There is no direct evidence that such a transmission could lead to clinical measles in seronegatives, although isolated cases without apparent contacts may be suggestive of such a mechanism. With an increasing fraction of vaccinated people, anti-MV titres in the general population tend to decrease [6] and the epidemiological relevance of SIR-susceptibles may increase. It is therefore important to understand the role of SIR-susceptibles for the epidemiology of measles in a world of global vaccination. We have observed SIR in parents exposed to children with measles during a recent outbreak [7]. Here, we characterize the SIR and we show that individuals with MV-specific IgG below a well defined level are likely to develop SIR.

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PATIENTS AND METHODS

Sera

With the support of the Direction de la Santé of the Ministry of Health, Luxembourg, a hot-line (00352-490648) for measles surveillance was installed in the Department of Immunology of the Laboratoire National de Santé (LNS). Between March and July 1996, an outbreak of measles occurred in the cantons of Wiltz and Clervaux in the Grand-Duchy of Luxembourg affecting at least 84 patients, mostly children. Single or paired sera were drawn from the measles patients and their parents by standard venupuncture after informed consent. An additional one to seven pre-exposure sera ($n = 110$) of each of 44 parents (36 mothers, seven fathers, one grandmother; average age 37·2 years, range 26·3–50·5 years, and one 67 years old) of children with measles were available from the LNS serum bank.

Haemagglutination inhibition assay

Triplicates of two-fold serial dilutions of sera (25 μl/well), were incubated in precooled V-shaped 96-well plates with 25 μl of a standard dose of purified MV (Edmonston strain, obtained from Dr Berbers, RIVM, Bilthoven, The Netherlands) and 25 μl containing 10^6 African Green Monkey erythrocytes (*Cercopithecus aethiops*; RIVM) [8]. All dilutions were done in PBS pH 7·4 containing 0·2% bovine serum albumin (BSA). Haemagglutination (HI) titre was defined as the highest dilution (after addition of virus) which completely prevented haemagglutination after 3 h at 37°C. The highest serum concentration tested was 1:22.

Neutralization assay

Two-fold serial dilutions of heat-inactivated serum in Dulbecco's modified Eagles' medium (DMEM) were preincubated in triplicates for 3 h at 4°C with 100 $TCID_{50}$/well MV. After adding 6×10^3 Vero cells/well the microtitre plates were incubated under tissue culture conditions. On day 5, virus infection was monitored. Serum concentrations refer to the serum dilution in the presence of MV, before Vero cells were added. Neutralization (NT) titre was the highest dilution which prevented virus infection in at least two out of three wells. The highest serum concentration tested was 1:32**.** Sometimes intermediate NT and HI titres were estimated.

ELISA

MV-specific IgG and IgM were measured with a commercial ELISA kit based on continuously MV-infected permanent simian kidney cells (Enzygnost; for MV-IgG or IgM purchased from Behringwerke, Marburg, Germany) following the manufacturer's instructions (serum dilution 1:231 for IgG and 1:42 for IgM**)**. For IgM determination, IgG was complexed with sheep anti-human IgG Fc fragment, which enhances the sensitivity and adsorbs rheumatoid factor. Net IgG or IgM (or ΔA) corresponds to the difference in mOD_{450} between infected and uninfected control wells. $\Delta A < 100$ mOD and > 200 mOD define negative and positive sera, respectively. Background levels were 80–110 (mean 87) mOD for IgG and 70–135 (mean 83) mOD for IgM.

To test MV-specific isotype subclasses, the IgG-specific conjugate was replaced by subclass-specific mouse monoclonals conjugated with biotin and alkaline phosphatase-conjugated streptavidin (ExtrAvidin-AP; all from Sigma, St Louis, MO). TBS with 1% Tween 20, pH 8, were used for all washing steps. Serum dilutions were 1:200 in TBS with 0·1% Tween 20 and 0·1% BSA pH 7·4. Sigma 104 served as a substrate and absorbance was measured at 405 nm after 45 min. Background levels were 100–200 mOD (IgG1–3), 500 mOD (IgG4).

Recombinant nucleoprotein (NP; gift from Dr F. Wild, Lyon, France) was coated overnight at 4°C to microtitre plates (Maxisorb, Nunc, Roskilde, Denmark) using 100 μl/well (0·8 μg/ml in TBS with 1% BSA and 0·1% Tween 20) and affinity-purified $F(ab')_2$ of goat anti-human IgG (γ-chain-specific) conjugated to alkaline phosphatase (1:500 dilution). Sera were tested at dilutions of 1:500.

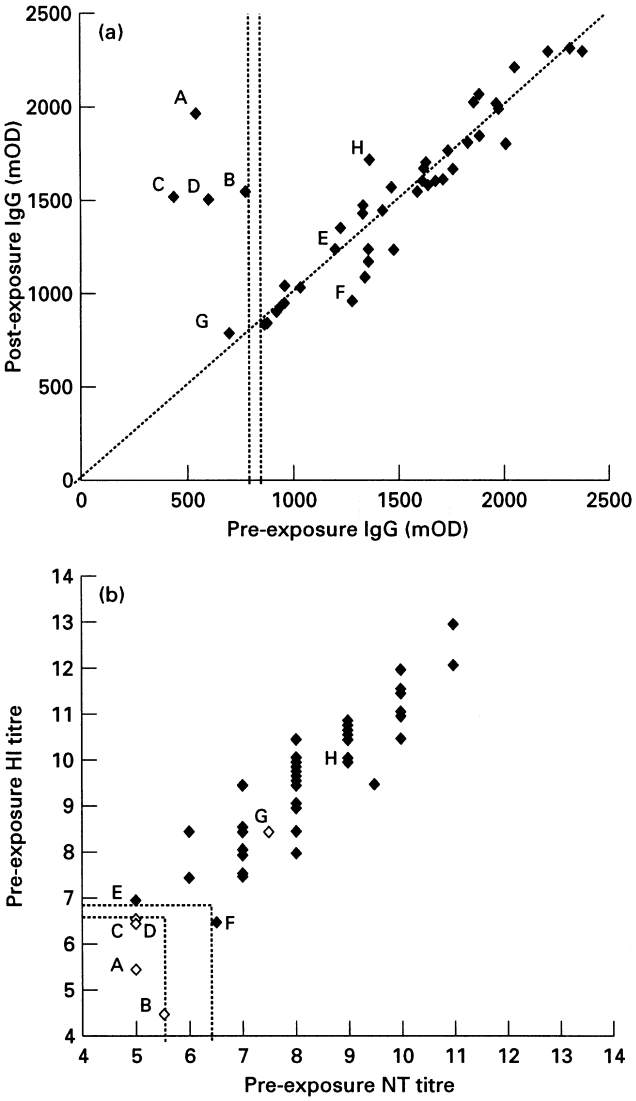

Fig. 1. (a) Correlation between pre- and post-exposure levels of measles-specific antibodies measured by IgG-ELISA (Enzygnost, Behringwerke) in paired sera of measles-exposed parents. The last serum available before exposure was used. - - - - -, IgG threshold level for secondary immune response (SIR; 780–870 mOD pre-exposure IgG) and the main diagonal. Absorbance was measured after 30 min, and is expressed as net IgG (see Patients and Methods). (b) Comparison of neutralization (NT) and haemagglutination inhibition (HI) titres of the last pre-exposure sera of all parents. Titres were measured as $\log_2$ dilutions. To avoid overlapping of symbols, identical values of HI were shifted by 0·1. Open and closed symbols correspond to individuals below or above the IgG threshold (850 mOD) as indicated in (a). - - - - - are drawn to exclude the lowest values of an individual without SIR and to include the highest value with SIR, to define NT and HI threshold titres for SIR (see text). Letters designate individual parents.

RESULTS

Pre- and post-exposure serology

The secondary immune response of parents from the children with measles was investigated during a recent outbreak. All children had clinical measles according to the Centers for Disease Control case definition [9] and developed specific IgG. Forty-six children were tested positive for IgM and showed a significant increase of specific IgG titres when paired sera ($n = 18$) were available.

Forty-four parents for whom pre-exposure sera were available were exposed while taking care of their children ($n = 51$) with measles. Some parents were exposed to up to four children with measles and none reported measles-related symptoms. All parents reported that they were not vaccinated and that they had had

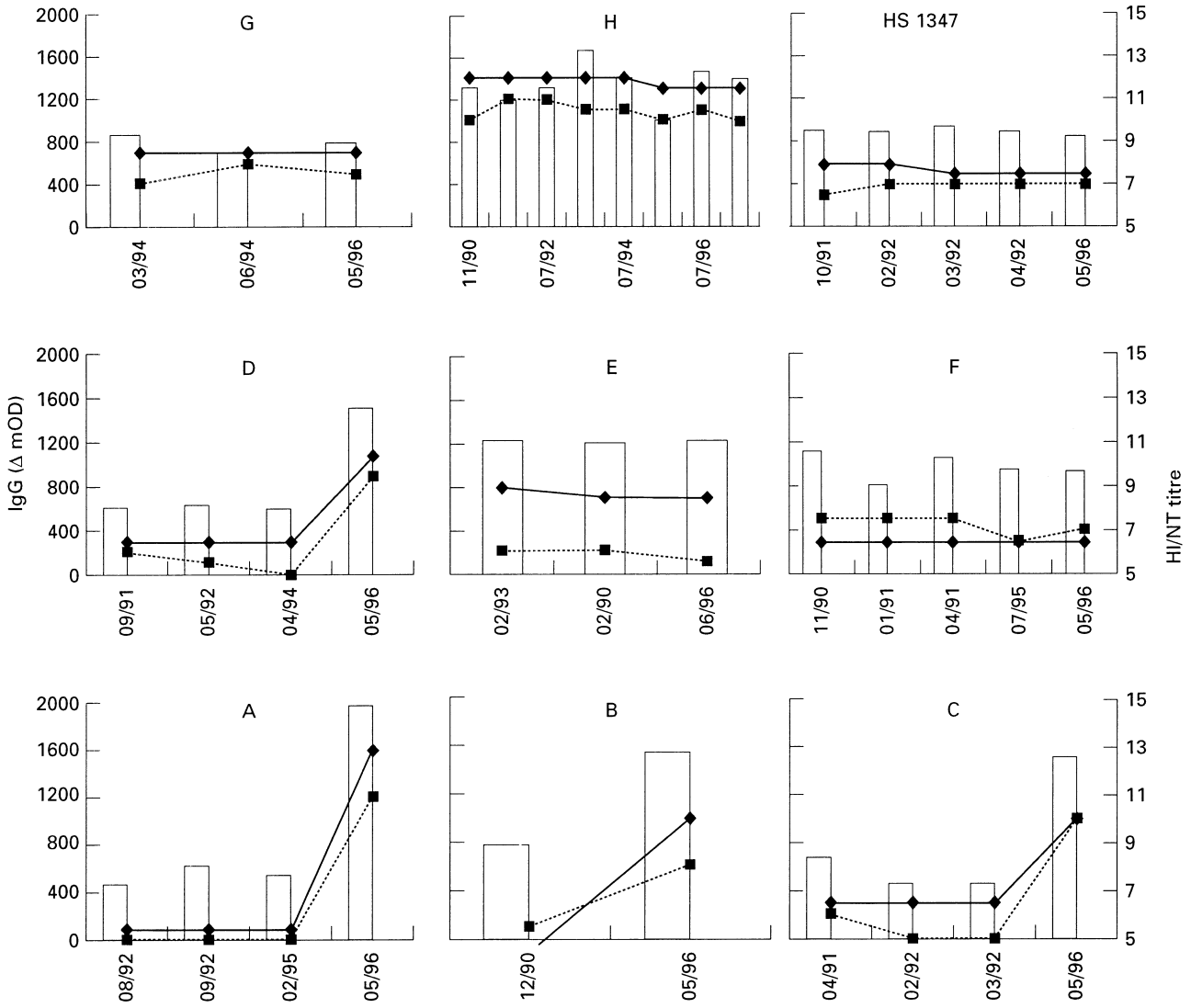

Fig. 2. Specific IgG levels (□), neutralization (NT) (....) and haemagglutination inhibition (HI) (————) titres of pre- and post-exposure sera of nine parents with the lowest pre-exposure anti-measles virus (MV) antibody levels, including individuals A–H (cf. Fig. 1). Dates of venupuncture are given along the abscissa, the last serum of each individual was drawn after MV exposure. For donor H two post-exposure sera are shown. Net IgG levels are expressed as mOD (left ordinate); HI and NT titres are expressed as $\log_2$ dilutions (right ordinate).

measles before entering school. The interval from the last pre-exposure sera to the exposure (first day of rash of the child) was 2–66 months. The post-exposure interval was between 2 and 12 weeks.

Before exposure, sera of all parents were clearly MV-IgG positive ($\Delta A > 400$ mOD). In Fig. 1a specific IgG reactivity of the last pre- and post-exposure IgG levels of all parents is shown. All but four parents exhibited no significant difference between pre- and post-exposure IgG levels. Above a pre-exposure level of 870 mOD, post-exposure antibody levels were < 150 mOD higher than pre-exposure titres (pre- *versus* post-exposure MV-IgG above 870 mOD pre-exposure absorbance: $P > 0{\cdot}5$, by paired *t*-test), except for one parent with considerable fluctuations in pre-exposure IgG levels (parent H in Fig. 2: $\Delta A = 300$ mOD) not seen in her corresponding NT or HI titres, or in any other individual. Below 780 mOD, measles antibodies of four out of five parents were boosted by 750–1400 mOD (pre- *versus* post-exposure MV-IgG below 780 mOD pre-exposure absorbance: $P < 0{\cdot}02$, by paired *t*-test). Pre-exposure titres of parents with (A,B,C,D) and without boosted antibodies were also significantly different ($P < 10^{-4}$).

This suggests that some parents developed SIR when re-exposed to measles. The individuals A, B, C and D were also lowest for their pre-exposure NT and HI titres (Fig. 1b). At pre-exposure titres of $\geq 1{:}2^{6{\cdot}5}$ for NT and $\geq 1{:}2^{7}$ for HI no significant booster effect was observed (pre- *versus* post-exposure titres: $P > 0{\cdot}1$ by paired *t*-test). Pre-exposure titres of SIR parents ($NT \leq 1{:}2^{5{\cdot}5}$; $HI \leq 1{:}2^{6{\cdot}5}$) were significantly lower than pre-exposure titres of parents without SIR ($NT \geq 1{:}2^{6{\cdot}5}$; $HI \geq 1{:}2^{7}$) (NT: $P < 10^{-12}$; HI: $P < 10^{-3}$ by non-paired *t*-test). The comparison of pre-exposure NT and HI titres of parents with and without SIR also shows that, while individual G was below the IgG threshold (780 mOD), she was well above the upper HI and NT threshold. Conversely, parents E and F were below the lower threshold for NT and HI, respectively, but not for IgG. Parents E, F, and G did not undergo SIR, suggesting that one parameter may not be sufficient to predict SIR.

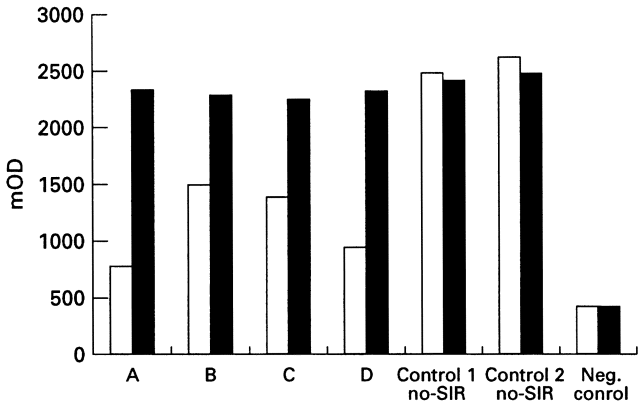

Fig. 3. IgG reactivity with recombinant nucleoprotein of donors with (A–D) and without secondary immune response (SIR) before (□) and after measles virus (MV) re-exposure (■). A MV-negative serum is shown as a negative control. All sera were diluted 1:500. Absorbance was measured after 4 h.

Thus, the thresholds below which susceptibility to SIR may be expected is defined by specific IgG < 780–870 mOD, NT $\leq 1{:}2^{5{\cdot}5}$ to $< 1{:}2^{6{\cdot}5}$, and HI $\leq 1{:}2^{6{\cdot}5}$ to $< 1{:}2^{7}$ using the protocols as described. These thresholds are undefined within the interval between the highest value (excluded) of an individual with SIR and the lowest value (excluded) of an individual without SIR.

Figure 1b also confirms that all parents were positive by *in vitro* neutralization (> 1:32) and by haemagglutination inhibition (> 1:22), i.e. at least for the lowest dilutions tested [7].

Figure 2 shows specific IgG, NT and HI titres of all available pre-exposure sera of the parents of Fig. 1 (including individuals A–H) with the lowest pre-exposure titres. In these sera or in any of the other parents' sera no significant changes in titre (< two-fold dilution) were observed during the 66 months before exposure. After measles exposure, the four parents A, B, C and D with IgG titres below the threshold (of 850 mOD) showed a significant and concomitant increase of NT (> four-fold increase, $P < 0{\cdot}005$ by paired *t*-test of pre- and post-exposure sera) and HI titres (> eight-fold; $P < 0{\cdot}006$; Fig. 2). None of the other parents (including E, F and G) showed a significant increase in post-exposure titres above a two-fold dilution ($P > 0{\cdot}1$). It is of interest to note that although IgG levels of the other parents shown in Fig. 2 were only a little above the threshold, these did not develop SIR by any of the parameters measured here.

Characteristics of parents with SIR

SIR occurred in mothers who were exposed to one or two children with IgM-confirmed measles. Pre- and post-exposure sera were drawn 14·2–64·0 months before and 4·6–6·0 weeks after exposure. None of the children was vaccinated. As all other parents, they reported having had measles before entering school. Although this was not independently confirmed by a physician, they were born at a time (1949–58) when immunity was acquired by early natural infection.

IgM and IgG subclasses during SIR

IgM and IgG subclasses participating in the SIR were investigated. Only low levels of IgM were found in three of the four SIR sera (A, 210; B, 84; D, 91). The thresholds for IgM provided in Patients and Methods are given for primary responses and are not necessarily valid for secondary responses.

Among the IgG subclasses, mainly IgG1 was boosted during the SIR. Two parents who were IgG4-negative, became positive (A, 810; B, 350 mOD) for this subclass after exposure, but this subclass showed elevated background values (500 mOD). IgG2 and IgG3 were essentially negative, before and after exposure.

Protein specificity of SIR

During natural measles infection, NP-specific antibodies are among the earliest and most abundant antibodies [10]. During SIR, NP antibodies increased concomitantly with HI and NT antibodies (Fig. 3).

Duration of booster effect

Thirty ± 1 week after MV re-exposure, 35–50% of the increase in specific antibody was already lost. HI and NT titres had decreased by two- to four-fold and two- to eight-fold, respectively. This demonstrates that the booster effect was only transient.

DISCUSSION

There are different manifestations after contact with wild-type measles, depending on the pre-existing immune status [5,11]. Seronegative people generally develop clinical measles. While seropositivity normally protects against disease, this study confirms that a SIR occurs in at least some protected individuals [12]. Among 44 fully protected, measles late convalescent parents re-exposed to measles, four developed an asymptomatic SIR with a significant increase in MV-specific IgG. Mainly the IgG1 subtype was boosted, which is also the predominant subclass during primary infection [13]. SIR included antibodies against MV-H (as measured by a concomitant increase in HI and NT) and against MV-NP (as detected by ELISA), which are also the main antibody targets during primary response [14]. SIR was sometimes associated with a weak IgM response, as reported before [15,16]. Higher and more transient increases of IgM may have been missed in sera which were drawn 4·6–6 weeks after exposure.

SIR was found only in individuals with a pre-exposure IgG level below a well defined threshold, which was determined in this study to be < 780–870 mOD. Above this threshold none of the parents developed a reaction to MV after exposure. SIR seems to be an 'all or none response', where the magnitude of increase in specific IgG is independent of pre-exposure antibody levels as long as these are below a certain threshold. Similar characteristics were also observed for NT and HI titres [7]. However, a single serological parameter may not be sufficient for the assessment of SIR susceptibility. The four parents with SIR (A–D) were the only individuals who were below the threshold for the three parameters, suggesting that only low specific IgG combined with low NT and HI titres predispose for SIR (Fig. 1).

Antibody levels tended to decrease again within 6–7 months after exposure. Antibodies persisted, however, long enough that an IgG increase would not have been missed in the other sera taken 4·6–6 weeks after exposure. This observation suggests that the low antibody levels found in these individuals were not caused by an insufficient MV contact, but rather by a low-responder status, which may be genetically defined, as has been suggested [17,18]. Consenting individuals with short-lived SIR could potentially be useful for the surveillance of circulating virus. A similar short-lived antibody increase was also observed in vaccinees after booster immunizations with live measles vaccine [19].

Several observations indicate that measles virus can circulate

among seropositive persons [4,5]. In a secluded population of seropositive vaccinees, most experienced an increase in measles titre several years after vaccination. In the absence of clinically overt measles, this suggested that the virus circulated also in healthy vaccinated individuals who could potentially transmit disease to seronegative people [5]. It is likely that this requires a transient viraemia. People undergoing a clinically inapparent SIR are the most likely candidates to support such a transmission of virus. In this context, the transient nature of the SIR seems to indicate that such individuals can be efficiently protected from disease, but not from infection. The frequency of SIR during measles outbreak was about 5% [12,20], but the number of susceptibles to SIR may be considerably higher. Our data can be used to estimate the frequency of susceptibles on the basis of their pre-existing IgG, NT and HI levels. Since after vaccination antibody titres are lower and more likely to wane than after wild-type infection [6,21–23], such estimates may become relevant to future MV epidemiology and vaccination strategies.

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