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Immunoglobulin G, A and M response to influenza vaccination in different age groups: effects of priming and boosting

By WALTER E. P. BEYER,* JOS T. M. VAN DER LOGT,†
RUUD VAN BEEK* and NIC MASUREL*

** Department of Virology and WHO National Influenza Centre, Erasmus University, Rotterdam, The Netherlands and † Department of Medical Microbiology, University of Nijmegen, The Netherlands*

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SUMMARY

Fifty volunteers, treated with an inactivated trivalent influenza vaccine containing A/Bangkok/1/79 (H3N2), A/Brazil/11/78 (H1N1) and B/Singapore/222/79 virus, were subdivided according to the estimated first exposure to influenza in their lifetime (priming) and the presence of antibodies against the vaccine components in the pre-vaccination sera. The isotypic antibody response (IgG, IgA, IgM) was determined by means of an antibody capture haemadsorption immunosorbent technique. For all three vaccine components, previously seropositive subjects produced antibodies of the IgG- and IgA-class more frequently than previously seronegative persons. Subjects primed to one of the influenza A subtypes showed more IgG and IgA responses in comparison with those unprimed (prime-effect). In contrast, IgM antibodies occurred in only 19 and 11 % of primed, but in 59 and 54 % of unprimed subjects, for A (H3N2) and A (H1N1), respectively. The incidence of IgM titre rises was not influenced by the prevaccination state. However, the mean magnitude of anti-A (H1N1)-IgM titre rises was greater in those previously seronegative. The concepts of primary and reinfection and of 'original antigenic sin' are discussed, and it is suggested that age and, if possible, serological state prior to antigen-exposure should be taken into account when studying isotypic antibody responses after influenza infection or vaccination.

INTRODUCTION

The annual administration of chemically inactivated influenza vaccine preparations remains an effective prophylaxis against epidemic influenza (Tyrrell & Smith, 1979), and is therefore recommended by health authorities in many countries for high-risk persons. Because of the very special epidemiology of influenza (constant antigenic drift, sudden occurrence of pandemic influenza A strains), the individual history of previous natural infections and vaccinations with sequential strains can be a factor influencing the effectivity of vaccination (Stuart-Harris & Schild, 1976). In particular, the first influenza A infection experienced during an individual's lifetime induces a potent immunological memory for the subtype to which the infective strain belongs ('priming').

Individuals primed by one subtype react differently to later homologous or heterologous infections or vaccinations than individuals primed for another subtype (the doctrine of 'original antigenic sin' (Francis, Davenport & Hennessy, 1953)). For instance, it could be shown that people born between 1939 and 1949 were most frequently primed by A(H1N1) strains closely related to those circulating again since 1977, whereas all other age groups were not (Masurel & André, 1978). Persons born after 1950 have been primed either to A(H2N2) or A(H3N2), two serologically cross-reacting subtypes (Dowdle *et al.* 1969). Those individuals primed by A(H2N2) strains, recall anti-H2N2-antibodies upon exposure to A(H3N2) (Masurel & Marine, 1973; Masurel, 1968).

This information has been confirmed by haemagglutination inhibition (HI) and, more recently, single radial haemolysis (SRH) techniques. However, few data are available on the reaction patterns of isotypic antibodies for prime- and booster-effects, especially of IgM, which is associated with primary exposure to a given antigen, and of serum IgA. With a new, recently described antibody capture method for haemagglutinating viruses (Van der Logt, Van Loon & Van der Veen, 1981), we have examined the pre- and post-vaccination sera of 50 individuals to study the correlation between the frequency and height of isotypic antibody response and previous history of exposure to influenza viruses as influenced by age and pre-vaccination antibody titres.

MATERIALS AND METHODS

Volunteers and vaccination schedule

In October 1980, 50 healthy volunteers aged between 15 and 53 years, living in The Netherlands, were vaccinated with an inactivated, trivalent influenza vaccine containing 10 μg haemagglutinin of A/Bangkok/1/79 (H3N2), A/Brazil/11/78 (H1N1) and B/Singapore/222/79 virus. None of the participants had been vaccinated against influenza before. Blood samples were collected prior to immunization (SI) and 28 days afterwards (SII).

Serologic studies

Sera were stored at −20 °C until use. Influenza viruses A/Bangkok/1/79 (H3N2), A/Brazil/11/78 (H1N1) and B/Singapore/222/79, kindly provided by Dr J. J. Skehel, WHO World Influenza Centre, London, England, were propagated in fertile hen's eggs.

Haemagglutination inhibition (HI) tests were done on sera pretreated with *Vibrio cholerae* filtrate by using standard techniques as previously described (Masurel, Ophof & De Jong, 1981). If no detectable titre was observed (< 9), it was arbitrarily recorded as 5 for subsequent calculations. Because of its lower avidity in the HI test, B/Singapore/222/79 virus was treated with ether according to Berlin *et al.* (1963).

Single radial haemolysis (SRH) tests were performed according to Schild, Pereira & Chakraverty (1975). Haemolysis on test plates was regarded as specific if no haemolysis occurred on control plates containing either complement and uncoated sheep erythrocytes or virus-coated erythrocytes without complement. Sera showing positive reactions on control plates as well were treated with packed sheep erythrocytes (1:5, v/v) for 1 h at 37 °C and re-tested.

The haemadsorption immunosorbent technique (HIT) was performed as described previously (Van der Logt, Van Loon & Van der Veen, 1981; Van der Logt *et al.* 1985). In short, sera were pretreated with guinea pig erythrocytes to absorb heteroagglutinins. Polystyrene U-form micro-haemagglutination plates (Thovadec Hospidex, Nieuwkoop, The Netherlands) coated with affinity purified goat antihuman IgM (μ-chain specific; Cappel Laboratories Inc., Conchranville, PA, USA) were incubated with serial two-fold dilutions of sera in HEPES saline albumin (HSA) buffer for 2 h at 37 °C. Serum controls were included. After washing the plates with tap water, four haemagglutination units of influenza antigen in 25 μl HSA buffer were added to each well except to the serum controls. After incubation overnight at 4 °C, 0·1 ml of a 0·07 % suspension of guinea-pig erythrocytes in HSA buffer was added. Plates were then incubated for 2 h at 4 °C and centrifugated at low speed for 10 min. A serum showing haemadsorption at a dilution of 1:320 or higher was considered positive, whereas a serum showing haemagglutination was considered negative. A positive and negative control serum were used in each test.

All serologic determinations were done twice. Results were expressed as the geometric (HI, HIT) or arithmetic (SRH) mean of the two experiments. Sera reacting negatively in both HI and SRH tests were regarded as seronegative (SI−); sera positive in at least one of both tests were regarded as seropositive (SI+).

Criteria for exclusion of subjects from the study

HI/SRH-response upon vaccination. An increase of pre- and post-vaccination titres of at least four-fold in the HI or at least 45 % in the SRH (Goodeve, Jennings & Potter, 1983) was regarded as a significant titre rise due to vaccination. Persons without this rise in both tests (nonresponders) were excluded from the study.

Discrepancy between HI/SRH and HIT. Volunteers showing negative HI and SRH titres but positive HIT titres in their pre-vaccination sera were excluded, as it was not possible to decide whether in these cases the HIT had given nonspecific results or had been more sensitive than the HI/SRH.

Criteria for exclusion were checked for each of the three antigens separately.

Calculations and statistics

Differences in frequency of qualitative variables (tested by χ^2 test) and in height of titres (tested by Wilcoxon-test) were regarded as significant when $P < 0{\cdot}05$.

RESULTS

Final study groups

Table 1 presents, for each antigen, the number of subjects excluded according to the criteria described in Materials and Methods. The remaining subjects were further subdivided according to state of their pre-vaccination sera, as determined by the HI and SRH techniques. Prior to immunization, a greater number of subjects were seropositive for A (H3N2) and B than for A (H1N1), probably reflecting the short period between the reappearance of the A (H1N1) subtype in 1977 and the start of the vaccination trial in 1980.

Table 1. *Number and state of pre-vaccination serum of subjects entering the study*

	Components of vaccine		
	A (H3N2)	A (H1N1)	B
Original number of vaccinees	50	50	50
Exclusions*			
Non-responders	2	3	2
Discrepancy between HI/SRH and HIT	—	3	3
Number of subjects entering the study	48	44	45
Pre-vaccination serum positive (SI+)*	34	22	35
Pre-vaccination serum negative (SI−)	14	22	10

* See criteria in Materials and Methods.

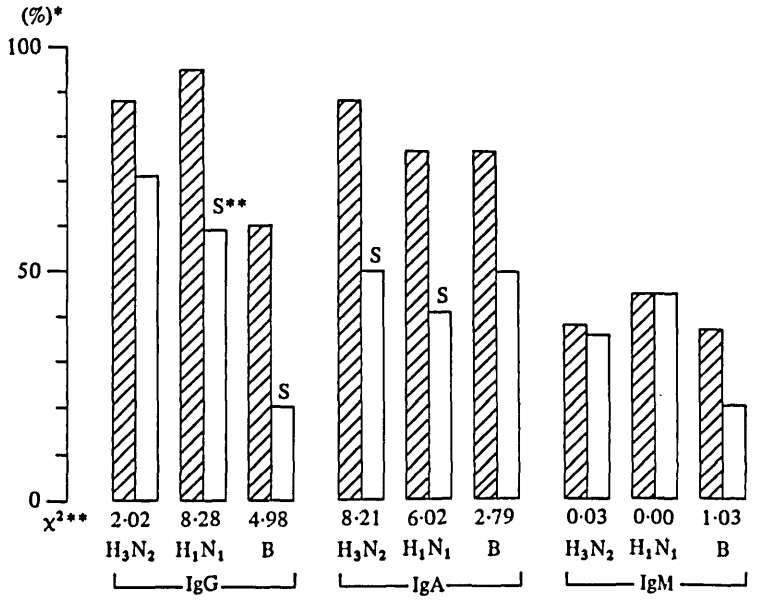

Fig. 1. Influence of previous seropositivity in prevaccination sera on titre rises of isotypic antibodies against vaccine components. ■, Previously seropositive (SI+). □, Previously seronegative (SI−). *, Subjects showing a titre rise of isotypic antibodies after vaccination (percentage, for total numbers see Table 1). **, χ^2-values of the differences between SI+ and SI−. Significant differences ($\chi^2 > 3{\cdot}84$, $P < 0{\cdot}05$) are marked by S.

Distribution of isotypic antibody responses according to pre-vaccination state

As shown in Fig. 1, subjects with positive HI/SRH-titres prior to vaccination obviously differ from previously seronegative subjects by producing an IgG- and IgA-response more frequently, e.g. 88 versus 50% for anti-A (H3N2)-IgA and 95 *v.* 59% for anti-A (H1N1)-IgG. These differences were all significant, except for anti-B-IgA (77 *v.* 50%) and anti-A (H3N2)-IgG (88 *v.* 71%). IgM responses occurred less frequently than IgG and IgA responses. The pre-vaccination state had no influence on the incidence of IgM titre rises, in contrast to IgG and IgA.

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Table 2. *Influence of priming on titre rises of IgG and IgA antibodies against A (H1N1) and A (H3N2) after vaccination*

Antibody-class	Subtype	Primed		Unprimed		Booster-effects	
		SI+	SI−	SI+	SI−	In unprimed*	In seronegatives†
IgG	A (H3N2)	18/21‡ (86%)	4/5 (80%)	12/13 (92%)	6/9 (67%)	+ (2·35)§	+ (0·28)
	A (H1N1)	3/3 (100%)	6/6 (100%)	18/19 (95%)	7/16 (44%)	+¶ (5·71)	+¶ (11·06)
IgA	A (H3N2)	19/21 (90%)	3/5 (60%)	11/13 (85%)	4/9 (44%)	+¶ (3·96)	+ (0·31)
	A (H1N1)	2/3 (67%)	3/6 (50%)	15/19 (79%)	6/16 (38%)	+¶ (6·22)	+ (0·28)

SI+, Pre-vaccination serum positive.

SI−, Pre-vaccination serum negative.

* Booster-effect in unprimed: difference in unprimed persons between those with positive and negative pre-vaccination serum. +, Higher frequency of isotypic titre rises in SI+ than in SI−.

† Prime-effect: difference between primed and unprimed persons with negative pre-vaccination serum. +, Higher frequency of isotypic titre rises in primed than in unprimed seronegatives.

‡ Number of subjects showing a titre rise of isotypic antibody/total number of subgroup (percentage in parentheses).

§ χ^2-values.

¶ Difference significant in χ^2 test ($P < 0{\cdot}05$).

Distribution of isotypic antibody responses according to priming

To examine possible stimulation of the immunological memory acquired during the first exposure to influenza in life, possibly many years ago, the subjects were further subdivided by age. All vaccinees were supposed to be primed by an influenza A subtype: persons born between 1939–49 were regarded as primed by A (H1N1), those of other ages as H1N1-unprimed. Persons born after 1950 had been primed by strains of two serologically related subtypes, A (H2N2) and A (H3N2). Older persons were regarded as H3N2-unprimed.

The distribution of IgG and IgA antibodies is given in Table 2. A booster-effect on recent infections without interference by possible prime-effects could be studied by comparing the unprimed groups. A positive boosting, i.e. a higher frequency of titre rises in previously seropositive than in seronegative subjects, occurred in both IgG and IgA classes and was significant except for anti-A (H3N2)-IgG ($\chi^2 = 2{\cdot}35$). A genuine prime-effect without disturbance by antibodies from possibly recent infections was deduced from a comparison between primed and unprimed seronegatives. The frequency of titre rises was higher in the primed SI− groups (significantly for anti-A (H1N1)-IgG). There were no quantitative differences in the mean magnitudes of titre rises between groups (shown for A (H1N1) in Fig. 2).

The pattern of IgM-antibodies was quite different. Primed persons scarcely produced an IgM response (see Table 3) but unprimed subjects did so in more than half the cases. Thus, a highly significant negative prime-effect could be demonstrated. Among the unprimed, no differences in number of titre rises

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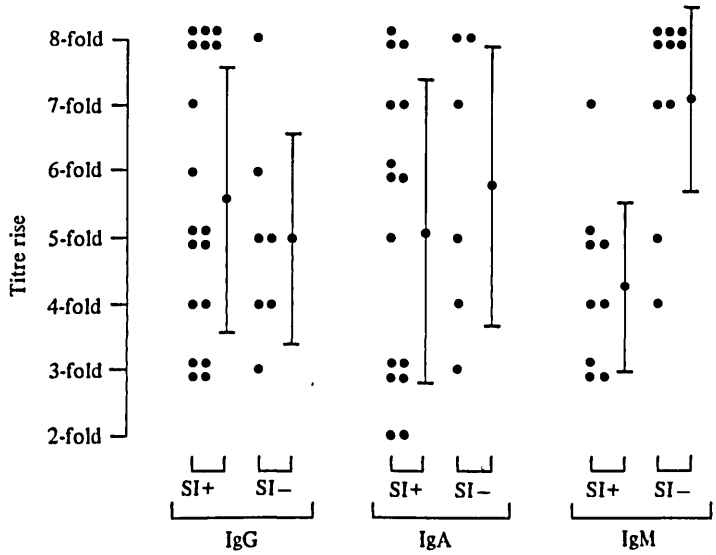

Fig. 2. Magnitude of titre rises of isotypic antibodies against A (H1N1) for subjects not primed by A (H1N1) measured by haemadsorption immunosorbent technique (HIT). Ordinate: factor of titre rise between pre- and post-vaccination sera. Negative pre-vaccination sera (< 320) were arbitrarily recorded as 80. ϕ, mean titre rise ± SD. For IgM, but not for IgG or IgA, SI+ and SI− differ significantly (Wilcoxon-test, $P < 0{\cdot}05$).

Table 3. *Influence of priming on titre rises of IgM antibodies against A (H1N1) and A (H3N2) after vaccination*

Subtype	Primed	Unprimed	Prime-effect*
A (H3N2)	5/26† (19%)	13/22 (59%)	— (8·08)‡
A (H1N1)	1/9 (11%)	19/35 (54%)	— (5·38)

—, Significantly less titre rises in primed than in unprimed subjects.
* Prime-effect: difference between primed and unprimed subjects.
† Number of subjects showing an IgM titre rise/total number of subgroup (percentage in parentheses).
‡ χ^2-values.

between previously seronegatives and seropositives was found. However, for A (H1N1) the mean magnitudes of IgM titre rises significantly differed for SI+ and SI− (Fig. 2), suggesting that the vaccine offered more new antigenic epitopes to previously seronegatives than to previously positives. For anti-A (H3N2)-IgM, this difference in the magnitudes of the titre rises could not be demonstrated.

Until now, the epidemiology of influenza B has not shown an antigenic shift. Therefore, four arbitrary age groups were studied (14–19, 20–30, 31–41 and 42–53 years of age). Apart from a non-significant decrease of IgG and IgA antibodies and increase of IgM antibodies in the oldest age group, no differences between age groups could be detected.

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DISCUSSION

Few trials have been undertaken to study the response of isotypic antibodies to inactivated influenza vaccine. While Leibenzon (1984) could detect a high IgG production, but no IgA or IgM response, using a rapid chromatography technique, others found both IgA and IgM antibodies in varying amounts (for reviews see Boyer, Cherry & Noble, 1977, and Gonchoroff *et al.* 1981). In the present study we observed that an inactivated influenza vaccine is able to stimulate antibodies of the IgA and IgM class under the right circumstances.

A variety of immunoassays have recently been used to study the occurrence and dynamics of isotypic antibodies after natural infection with influenza A and B viruses (for reviews see Julkunen, Pyhälä & Hovi, 1985; Döller, Döller & Gerth, 1985; Goldwater, Webster & Banatvala, 1982) to evaluate their possible value in rapid diagnosis. Seroconversions of IgG and IgA antibodies were usually observed in a high proportion of cases. When subjects with obvious primary infections were studied, IgM was also induced in most cases (Murphy *et al.* 1982; Burlington *et al.* 1983). However, in study groups with a broad age distribution or not distinctly defined with respect to age, IgM antibodies were significantly less frequently observed than IgG and IgA. For instance, Julkunen *et al.* (1985) found an IgG1 response in 100% of persons with a confirmed A (H3N2) infection and an IgA1 response in 60% but an IgM response in only 30%. This agrees with the findings for our vaccination group as a whole: IgG 83%, IgA 77%, IgM 38%. The low IgM response rate has been explained by the supposition that most of the individuals tested had experienced secondary influenza virus infections and that the antigenic drift of the infective strain was not sufficient to cause IgM antibodies against the new epitopes on the HA molecule (Burlington *et al.* 1983). However, to our knowledge, no attempt has been made before to define more clearly the individual history of previous exposure to influenza viruses, especially in view of the phenomenon of 'original antigenic sin' (priming).

We have tried to find an association between the production of isotypic antibodies of the IgG-, IgA- and IgM-class after influenza vaccination, using age as a measure of priming and the presence of antibodies against the vaccine components in pre-vaccination sera of unprimed subjects. As none of the volunteers had previously been vaccinated against influenza, the seropositive subjects (SI+) had acquired their antibodies by natural infection. The seronegatives were also assumed to have been exposed to influenza, since young children were not included in this study.

The previous exposures to influenza antigens influenced the antibody production after vaccination. We were able to distinguish two different booster-effects: the genuine boosting of recent infections, and the prime-effect which reflects the first infection in life. Both booster-effects showed similarities, in the sense that previously seropositive as well as primed subjects more frequently produced IgG and IgA antibodies after vaccination as compared to the complementary groups. Furthermore, in contrast to IgG and IgA, the prime-effect was characterized by a virtual absence of an IgM response in primed subjects but a substantial IgM response was found in unprimed subjects. No booster-effect for IgM on recent infections was observed. Because of the extreme heterogeneity of the influenza

viruses in time and place, total identity of the viral antigenic structures between two consecutive epidemics of the same subtype or between vaccine components and epidemic strains is nearly impossible. On the other hand, among closely related variants, only a few changes in the amino-acid sequence of the antigenic proteins can be seen; the rest of the molecules remain unchanged (Wiley, Wilson & Skehel, 1981). Thus, every new exposure to influenza may give rise to both a primary reaction including IgM antibodies against the truly new antigenic structures and a secondary reaction against the already experienced epitopes of the virus. In previously seropositive persons, the number of newly encountered epitopes may be small and result in low IgM titres, whereas there is a greater chance that in seronegatives a greater number of epitopes never previously encountered will cause the production of more IgM antibodies of different specifity and a higher total IgM concentration. This has been found with anti-A (H1N1)-IgM in unprimed subjects. These findings should be taken into account in the development of rapid diagnostic techniques for the diagnosis of influenza based on the presence or absence of specific IgM in a single serum (Burlington *et al.* 1983; Van der Logt *et al.* 1984).

Overall, booster-effects turned out to be more significant for A (H1N1) than for A (H3N2) probably because the priming period was better defined for A (H1N1). In this study, persons who were included in the H3N2-primed group (born after 1950) had possibly been primed by the related H2N2 subtype. The results for influenza B could serve as a control because major different subtypes and hence the basis for 'original antigenic sin' does not exist to our knowledge. Indeed, no major differences between age groups could be seen but there was a distinct booster-effect from recent infections (Fig. 1).

Our results agree with those from the vaccination trial made by Boyer, Cherry & Noble (1977) who found a correlation between the age of vaccinees and the occurrence of specific IgM antibodies after vaccination with the influenza A/New Jersey/76 strain. They also agree with the animal model of Gonchoroff *et al.* (1981) who used a solid-phase fluorescence immunoassay to detect IgG and IgM antibodies. Mice were primed with an A (H1N1) virus (infection), and boosted with either the homologous virus or a different A (H1N1) strain or a strain of the A (H2N2) subtype (vaccination). Whereas IgG directed against the priming virus was recalled not only by re-exposure to the homologous virus but also by exposure to the other strains (original antigenic sin), no change in specific IgM levels was observed. Only IgM specifically directed against the heterologous viruses was induced.

In conclusion, both age and serologic state prior to exposure may influence isotypic antibody responses to influenza viruses and should be included in the evaluation of the effects of natural infection or vaccination.

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