Clin. exp. Immunol. (1989) **78**, 201–206

Immune adherence of nascent hepatitis B surface antigen–antibody complexes *in vivo* in humans

N. MADI, J. P. PACCAUD, G. STEIGER & J. A. SCHIFFERLI *Clinique Médicale and Division of Nephrology, Department of Medicine, Hôpital Cantonal Universitaire, Geneva, Switzerland*

(Accepted for publication 10 July 1989)

SUMMARY

Upon i.v. injection into humans, pre-formed immune complexes bind complement and adhere to complement receptor type 1 (CR1, CD35) on erythrocytes (immune adherence). However, in most circumstances antigen and antibody react in the presence of complement; such nascent immune complexes may have properties different from pre-formed immune complexes. To define whether nascent immune complexes would also adhere to erythrocytes *in vivo* in humans, we studied immune complexes that formed upon i.v. injection of radiolabelled hepatitis B surface antigen (HBsAg) into immunized volunteers (eight subjects with anti-HBsAb levels ranging from undetectable to 50 U/ml.; and three control non-immune individuals). Immune complexes formed immediately in the subjects with detectable levels of specific antibody, and the clearance rate of these immune complexes correlated with the anti-HBsAb level ($\tau = 0{\cdot}78$, $P < 0{\cdot}01$). A fraction of the circulating immune complexes bound to erythrocytes in the three individuals with the highest antibody level (8–15% at 10 min). The effect of CR1 number per erythrocytes was analysed in two subjects with similar antibody levels and immune complexes clearance rates: immune adherence was higher in the subject with more CR1 per erythrocytes. The same immune complexes model studied *in vitro* provided similar results: a fraction of nascent immune complexes bound to human erythrocytes; this immune adherence was observed only when immune complexes formed in the presence of antibody excess, and correlated with CR1 number per erythrocytes ($r = 0{\cdot}99$, $P < 0{\cdot}01$). Finally, adherence of nascent HBsAg–antibody immune complexes to platelets was demonstrated in rabbits. Although immune adherence involves only a small fraction of nascent immune complexes at any given time, it may be essential for the safe disposal of large nascent immune complexes.

Keywords nascent immune complexes immune adherence complement receptor type 1 (CR1, CD35) hepatitis B surface antigen

INTRODUCTION

Recent evidence suggests that complement and the C3b receptor (CR1, CD35) on erythrocytes are involved in the transport and processing of immune complexes in the circulation. In experiments performed in monkeys, it has been shown that large pre-formed immune complexes injected intravenously fix complement and bind to CR1 on erythrocytes (Cornacoff *et al.*, 1983; Waxman *et al.*, 1984; 1986). A similar sequence of events has been described in the circulation in humans after the i.v. injection of pre-formed tetanus toxoid/anti-tetanus toxoid immune complexes (Paccaud *et al.*, 1987; Schifferli *et al.*, 1988; 1989). Immune adherence may prevent the deposition of immune complexes in organs outside the fixed macrophage system (Waxman *et al.*, 1986).

Correspondence: Dr J. A. Schifferli, Clinique Médicale, Hôpital Cantonal Universitaire, 1211 Geneva 4, Switzerland.

However, under most circumstances, immune complexes form directly in the presence of complement whether the antigen–antibody reaction occurs in or outside the circulation. Such nascent immune complexes react immediately with complement and they acquire unique properties, i.e. they remain soluble (Schifferli *et al.*, 1985) and they have a reduced (or absent) capacity to adhere to human erythrocytes (Schifferli & Peters, 1983; Varga, Thiry & Füst, 1988; Paccaud, Steiger & Schifferli, 1989). However, this is not a general rule, and some nascent immune complexes have been shown to react with complement receptors on circulating cells. In particular, it has been shown that nascent DNA–anti-DNA complexes adhere to erythrocytes in monkeys and to platelets in rabbits (Edberg, Kujala & Taylor, 1987) [platelets bear the immune adherence receptor in rabbits (Taylor *et al.*, 1985)]. It is not known whether nascent immune complexes formed in the circulation in humans would bind to erythrocytes.

In this study we analysed the sequence of events that takes place when immune complexes are formed directly *in vivo* by the

injection of radiolabelled hepatitis B surface antigen (HBsAg) into immunized individuals. Initial experiments performed *in vitro*, and *in vivo* in rabbits, indicated that a small fraction of nascent immune complexes may become immune adherent in humans. This is what we observed in the three individuals with the highest level of antibody to HBsAg (anti-HBsAb).

MATERIALS AND METHODS

Antigen, antibody and CR1 measurements

HBsAg was kindly provided by Dr P. Adamovicz (Pasteur Institute, Paris). The HBsAg (mol. wt 3 000 kD) was dialysed against phosphate-buffered saline (PBS), pH 7·4. The HBsAg was radiolabelled with the Bolton and Hunter reagent (*N*-succinimidyl-3-(4-hydroxy,5-[^{125}I] iodophenyl)propionate) (Amersham International, Amersham, UK) to a specific activity of 2·3 μCi ^{125}I/μg HBsAg (Bolton & Hunter, 1973). The preparation was sterile and pyrogen-free when tested in two rabbits. Penicillin G (250 000 U/ml) and streptomycin (2·5 mg/ml) were added, and aliquots were stored at 4°C for no more than 4 weeks. Sera were obtained from recently immunized individuals; their anti-HBsAb level was measured by a solid-phase ELISA [Ausab® EIA, Abbott Laboratories, Zug, Switzerland).

Erythrocyte CR1 numbers were measured as previously described (Ross *et al.*, 1985) using a mouse monoclonal anti-CR1, E11 (kindly provided by Dr N. Hogg) radiolabelled to a specific activity of 0·47 μCi/μg using the iodogen method (Fraker & Speck, 1978).

In vitro *immune adherence assay*

Human erythrocytes. This assay was performed in two steps: first, nascent immune complexes were formed in the presence of normal human serum using 50–76 ng of ^{125}I–HBsAg, and 12·5–50 μl of immune sera. The mixtures (final volume 100 μl in PBS) were incubated at 37°C. Controls included EDTA (10 mM) chelated mixtures and serum devoid of anti-HBsAb activity. Then, after various periods of time (when not indicated, 15 min), aliquots (15 μl) were removed and added to 300 μl of erythrocytes suspended to an haematocrit of 5%, in PBS–bovine serum albumin (BSA) 1% w/v. After incubation for 15 min at room temperature, the cells were layered on 200 μl of dibutylphtalate/dinonylphtalate (4/1, v/v) in 400-μl capacity Eppendorf tubes, and centrifuged at 10 000 *g* for 30 sec. The tubes were then clipped, and pellets and supernatants counted for radioactivity. Controls included trypsin-treated erythrocytes (Paccaud *et al.*, 1987). All assays were done in duplicate.

Human erythrocytes and rabbit platelets, using whole blood. Preliminary experiments have shown that complement activation was slightly delayed, but not blocked in whole blood chelated with 1/10 citrate (sodium citrate $2H_2O$, 31·3 g/l). Radiolabelled HBsAg (165 ng) was added into 300 μl of citrated whole blood and incubated for 15 min at 37°C. The cells were washed twice with ice-cold PBS by centrifuging at 10 000 *g* at 4°C for 10 min. The experiments were performed using immune or non-immune whole blood; controls included assays performed in the presence of 10 mM EDTA, and using citrated plasma instead of whole blood. All assays were done in duplicate. The percentage of counts pelleted in non-immune blood, in blood chelated with 10 mM EDTA and in citrated plasma was always below 5·8% in rabbits and 2·5% in humans. Thus binding below these values was considered as non-specific for immune adherence.

Erythrocyte labelling with ^{99m}*Tc*

Erythrocytes were labelled under sterile conditions with ^{99m}Tc *in vitro* using gluco-scintimed solution (stannous gluco-ene-diolate) (Banna Laboratories, Geneva, Switzerland). Whole blood (1·25 ml) was first incubated with 25 μl of gluco-scintimed solution for 5 min at 22°C with gentle intermittent shaking. The mixture was then washed once with 10 ml of sterile isotonic saline before adding 100 μCi of ^{99m}Tc. After an incubation of 10 min at 22°C, the erythrocytes were washed three times with 10 ml saline before use.

Studies in rabbits

Two white rabbits were immunized with HBsAg (10 μg) once in Freund's complete adjuvant (FCA) and twice in Freund's incomplete adjuvant (FIA) at 2-week intervals. The experiments were carried out 3 weeks after the last immunization. Two non-immune rabbits served as controls. Each rabbit was injected intravenously with 950 ng of ^{125}I–HBsAg that had been mixed with a 500 μl suspension of autologous ^{99m}Tc-labelled erythrocytes (5·8–10·9 μCi) as a bolus; ^{99m}Tc-labelled erythrocytes facilitated accurate estimation of blood volume. Blood samples of 600 μl were drawn into EDTA after various time intervals. Platelets were then separated from blood samples by processing immediately at 4°C; 500-μl samples were centrifuged at 10 000 *g* for 10 min at 4°C, conditions shown to be sufficient to separate the platelets (> 95% in the pellets) from the plasma. The pellets were washed once in PBS at 4°C. ^{99m}Tc and ^{125}I activity in the samples was measured using a twin-channel gamma-counter. The sum of the first supernatant and the PBS wash was taken to represent total plasma radioactivity.

Human studies

These studies were approved by the Ethical Committee of the Department of Medicine, Hôpital Cantonal Universitaire, Geneva. The normal volunteers included eight individuals (five men, three women) who had been immunized against hepatitis B between 6 and 3 months previously, and three non-immune individuals (2 men and one woman). They were aged between 22 and 30 years old. Specific anti-HBsAb levels in the immune subjects were, respectively: undetectable (< 0·01 U/ml), 0·033, 0·32, 0·38, 0·4, 28·6, 34·7 and 50 U/ml. CR1 numbers per erythrocytes in the three subjects with the highest antibody level were, respectively 601, 487 and 186.

Two to four hours before the injection of the antigen, the subjects were tested for immediate hypersensitivity reaction by an intradermal injection of 50 μl of a HBsAg suspension (1 : 1000 in 0·9% NaCl). All subjects received 1·5 ml of 5% Lugol solution at least 3 h before the injection and then three times a day for 48 h. They were injected intravenously in less than 5 sec with a mixture containing 2 μg of ^{125}I–HBsAg and 8–20 μCi of ^{99m}Tc-labelled autologous erythrocytes. Blood samples were drawn from an indwelling canula in a peripheral vein in the opposite arm, at different time intervals and immediately processed at 4°C, as above. Radioactivity was measured in samples of whole blood (2 ml) and of erythrocytes that had been washed twice with 10 ml of cold PBS. TCA-precipitable counts were determined in each blood sample.

Demonstration of immune complex formation in vivo
Sucrose density gradient ultracentrifugation (SDGU) (Schifferli *et al.*, 1985) (10–50% w/w, 35 000 rev/min for 3 h) was performed on plasma samples obtained at 1, 2, 4, 6 and 8 min after injection. Each run included free ^{125}I-HBsAg. The appearance of radiolabelled antigen in larger mol. wt fractions was considered as indicative of immune complex formation.

Samples were also analysed by affinity chromatography on protein A (protein A-Sepharose, Pharmacia Fine Chemicals, Uppsala, Sweden). Preliminary experiments demonstrated that more than 80% of the radioactivity was recovered in the effluent when ^{125}I-HBsAg was mixed with non-immune serum before being applied to protein A-Sepharose. By contrast, less than 10% was recovered in the effluent when ^{125}I-HBsAg was first mixed with immune serum, indicating that immune complexes had formed.

Assessment of immune adherence and immune elimination in vivo
In rabbits and humans, the immune adherence was calculated as follows: percentage immune adherence = [^{125}I counts recovered in the pellet (containing platelets in rabbits, and erythrocytes in humans) × 100]/TCA precipitable ^{125}I counts present in whole blood. As previously reported (Schifferli *et al.*, 1988), the initial elimination rate of ^{125}I-HBsAg (until 4 min) was determined using the ^{125}I:^{99m}Tc ratios as follows: elimination rate (in percentage) = [(TCA-precipitable ^{125}I:^{99m}Tc ratio in the whole blood) × 100]/(TCA-precipitable ^{125}I:^{99m}Tc ratio of the sample injected). The elimination rate in later samples was directly computed from the rate of clearance of TCA-precipitable ^{125}I from whole blood, using the 4-min sample as the reference value.

Statistical analysis
When appropriate we used the linear or the Kendall rank correlation coefficients (r and τ).

RESULTS

Immune adherence of nascent HBsAg–HBsAb complexes in vitro
Three of the main factors that would influence immune adherence in the circulation in humans were assesed *in vitro*. First, the kinetics of the reaction were followed when HBsAg was added to immune serum. The formation of immune complexes was immediate, as measured by retention of the antigen by protein A-Sepharose (see Materials and Methods). The nascent immune complexes formed acquired the capacity to bind to erythrocytes after 2 min, and this capacity became maximal at 15 min (Fig. 1a). There was no increase in binding at 30 and 60 min (not shown). Second, the antibody level influenced the immune adherence reaction (Fig. 1b); immune adherence was demonstrated only in sera containing high levels of anti-HBsAb, although immune complexes were formed in the serum with the lowest antibody level, as assessed by retention of the antigen by protein A-Sepharose. The equivalent point, which corresponded to 300 ng antigen for 1 U of antibody, would have been reached in a serum containing 3·3 U/ml under the experimental conditions of Fig. 1b. Thus, immune adherence was observed only for immune complexes formed in antibody excess. Third, the level of immune complexes binding at 15 min correlated directly with the CR1 number per erythrocytes (Fig. 1c) ($r = 0{\cdot}99$, $P < 0{\cdot}01$), although reactions were performed in large erythrocyte excess.

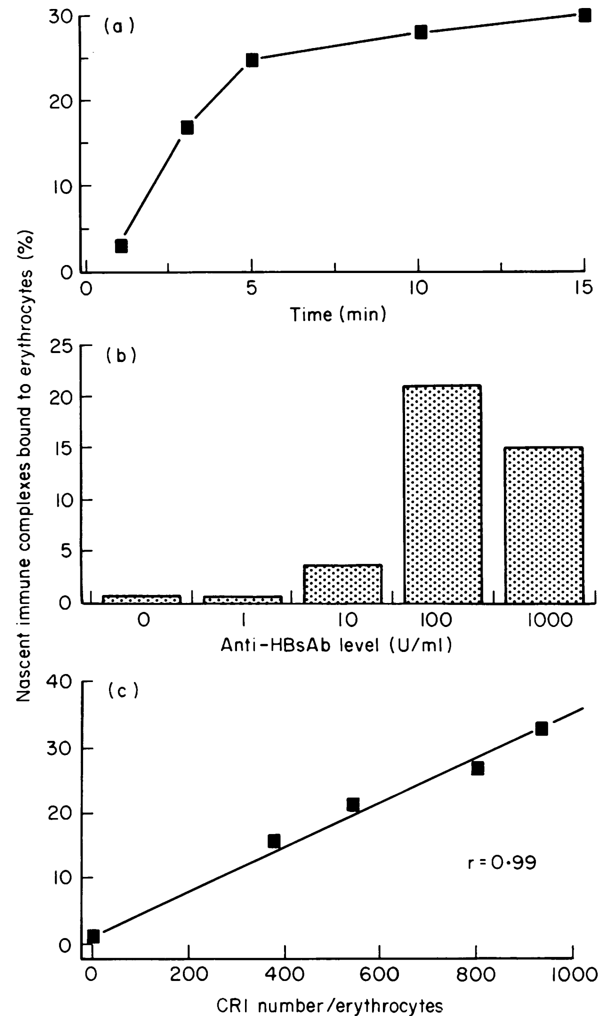

Fig. 1. Immune adherence of HBsAg–Ab complexes *in vitro* in humans. (a) Kinetics of the formation of immune complexes that bound to erythrocytes: ^{125}I-HBsAg was added to normal serum containing 100 U/ml of anti-HBsAb and incubated for various periods of time at 37°C; aliquots were removed and mixed with a suspension of erythrocytes (5% in PBS–BSA 1%). The proportion of immune complexes bound to erythrocytes was measured after an incubation time of 15 min at 22°C. (b) Immune adherence as a function of specific antibody levels: the HBsAg was added to human sera containing different anti-HBsAb levels and incubated for 15 min at 37°C. The proportion of immune complexes bound to erythrocytes was determined as above. (c) Immune adherence as a function of CR1 number per erythrocytes: the HBsAg was added to normal human serum containing 100 U/ml of specific antibody and incubated for 15 min at 37°C. Aliquots were mixed with erythroctyes bearing different numbers of CR1, and the proportion of immune adherent immune complexes determined as above (trypsinized erythrocytes served as negative controls).

Immune adherence and elimination of nascent HBsAg–HBsAb complexes in vivo *in rabbits*
When injected into two non-immune rabbits, the ^{125}I-HBsAg was cleared at a slow rate (34 and 40%, in 2 h) (Fig. 2a) although a small fraction of the HBsAg disappeared immediately (9 and 14%). The HBsAg remained free in plasma (Fig. 2b).

The fate of ^{125}I-HBsAg was very different in the two immune animals. A fraction (up to 38% in one rabbit) of the circulating

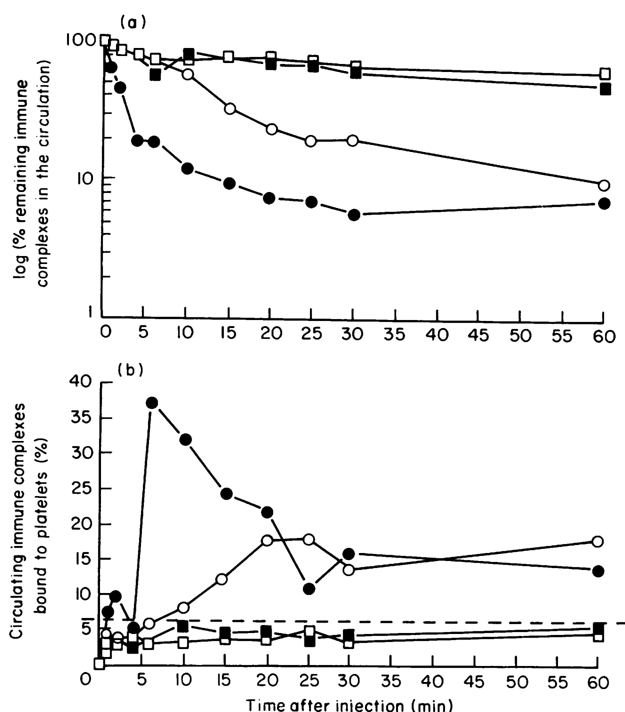

Fig. 2. Elimination (a) and platelet binding (b) of circulating ^{125}I-HBsAg in immune (circles) and non-immune (squares) rabbits. The two immune rabbits had, respectively, 3·3 U/ml (●) and 0·45 U/ml (○) of specific anti-HBsAb. Values below the dashed line (5·8%) are not specific for immune adherence (see Materials and Methods).

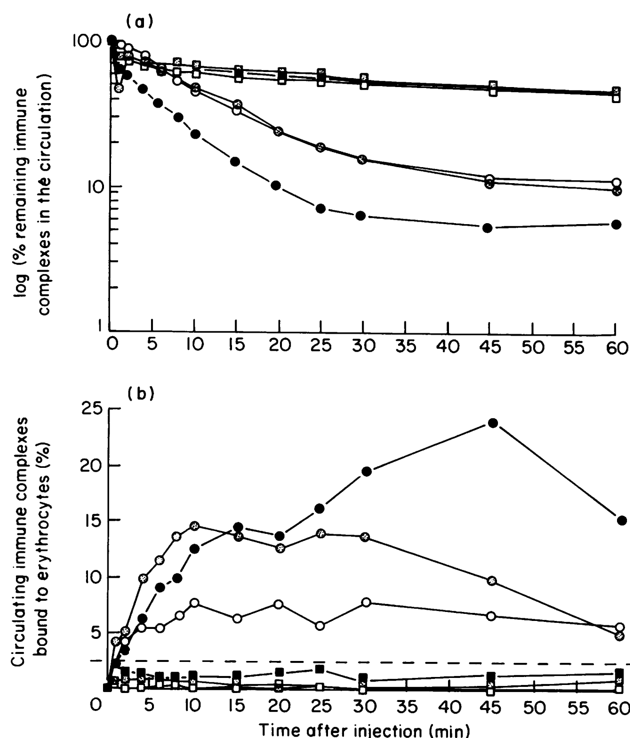

Fig. 3. *In vivo* assays in humans. Elimination of circulating ^{125}I-HBsAg (a) and binding of immune complexes to erythrocytes (b) in three non-immune (squares) and three immune subjects (circles). The three immune subjects shown are those with the highest antibody level: ● = 50 U/ml (CR1 = 186 receptors/erythrocytes); ○ = 34·7 U/ml (CR1 = 487); ⊗ = 28·6 U/ml (CR1 = 601). The presumed effect of CR1 number/erythrocytes on immune adherence was assessed by comparing the two individuals with similar immune complexes elimination rates and antibody levels i.e. ○ and ⊗. Values below the dashed line (2·5%) are not specific for immune adherence (see Materials & Methods).

protein-bound radioactivity was recovered in the pellets after centrifugation of the blood samples. Presumably, this HBsAg was incorporated into immune complexes that had fixed complement and attached to platelets. This sequence of events was suggested by the following results. First, immune complexes formed immediately *in vivo*. The HBsAg remaining in plasma was shown to be complexed by rabbit IgG: when samples taken at 1 and 6 min were analysed by SDGU, the position of the HBsAg had shifted towards higher mol.wt fractions, indicating that it was incorporated into a macromolecular complex. In addition, more than 90% of the labelled antigen in the same samples was retained by protein A-Sepharose. Second, that nascent HBsAg–HBsAb complexes fixed complement and bound to platelets *in vivo* was suggested by experiments performed *in vitro*. When HBsAg was added into citrated blood of an immune rabbit and incubated for 10 min at 37°C, a fraction of the nascent immune complexes bound to platelets (i.e. between 10 and 15% of the radioactivity was recovered in the pellets). By contrast, the pellets of control experiments contained always < 5·8% of the radioactivity (see Materials & Methods).

As expected, nascent HBsAg–HBsAb complexes were eliminated more rapidly than was free antigen, and this clearance was faster in the rabbit with the higher antibody level.

Immune adherence and elimination of nascent HBsAg–HBsAb complexes in vivo *in humans*

When injected into three non-immune subjects, the HBsAg was cleared slowly (between 40 and 50% in 1 h) although a fraction disappeared immediately (up to 25%) (Fig. 3a). This clearance pattern was similar to that observed in non-immune rabbits. As expected, there was no evidence of immune complexes formation (Fig. 4a), and the free HBsAg did not bind to erythrocytes (Fig. 3b)

In immune subjects with a detectable level of anti-HBsAb, the antigen was immediately incorporated (at 1 min) into immune complexes. In the three individuals with the highest antibody levels, there was an immediate shift on the SDGU of ^{125}I-HBsAg (30S) towards very large molecular weight fractions indicating the formation of immune complexes in plasma samples taken up to 8 min after injection (Fig. 4b); this shift was less marked in the other subjects with lower antibody levels. In all seven individuals with detectable levels of specific antibody, more than 90% of the ^{125}I-HBsAg in the samples taken at 1 min were retained by protein A-Sepharose.

A fraction (up to 24%) of circulating immune complexes bound to erythrocytes in the three individuals with the highest anti-HBsAb level (Fig. 3b), but not in the others in whom no specific binding could be demonstrated (< 2.5%; see Materials & Methods). The percentage of circulating immune complexes that were bound to erythrocytes increased during the first minutes. The percentage of immune adherence could be compared in the two subjects with almost identical antibody levels

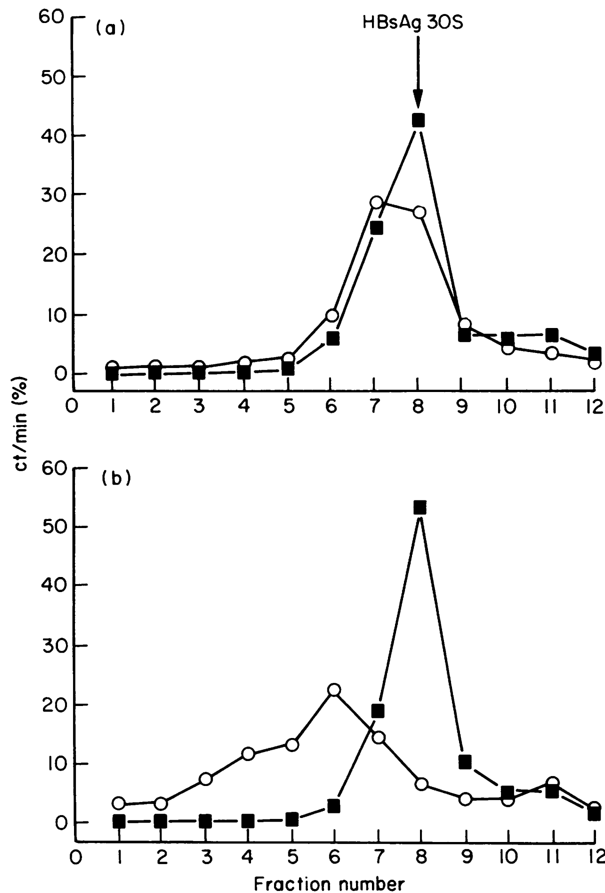

Fig. 4. Demonstration of immune complexes formation by SDGU *in vivo* in humans. Size of ^{125}I-HBsAg in a non-immune (a) and an immune (b) subject 4 min after i.v. injection of the antigen (○). Control: free ^{125}I-HBsAg (■). The ^{125}I-HBsAg shifted to higher mol. wt fractions in the immune individual with an anti-HBsAb level of 50 U/ml. Bottom of the gradient on the left.

(28·6 and 34·7 U/ml), immune complexes size and elimination rates. Maximal immune adherence was higher (15 *versus* 8%) in the subject with the higher number of CR1 per erythrocytes (601 *versus* 487) (Fig. 3b).

The elimination of the HBsAg (percentage cleared at 30 min) correlated directly with the size of the immune complexes formed ($\tau = 0{\cdot}69$, $P < 0{\cdot}002$), and with the specific antibody levels ($\tau = 0{\cdot}78$, $P < 0{\cdot}01$).

DISCUSSION

The data presented here provide the first demonstration that, in the circulation of humans, nascent immune complexes bind to erythrocytes.

HBsAg was used for several reasons. The antigen is well characterized and used in human immunization; thus it is safe for experimentation in humans. The elimination rate of HBsAg from the circulation is slow (< 50% in 1 h) and can be monitored easily. This immune complexes model could be initially analysed *in vitro*, and *in vivo* in rabbits. Lastly, many reports suggest that HBsAg–HBsAb complexes may circulate in the blood stream and deposit in tissues where they might be responsible for inflammation (Gocke *et al.*, 1970; Trepo *et al.*, 1974; Hirschel *et al.*, 1977).

In the three immunized subjects with the highest anti-HBsAb levels, the injection of HBsAg was followed by a sequence of events similar to that seen in immunized rabbits: immune complexes formed rapidly, and a fraction of the circulating immune complexes became adherent to cells bearing CR1, i.e. erythrocytes in humans. *In vitro* data indicated that complement-independent binding of immune complexes was negligible.

A series of observations merit attention. First, the immune adherence reaction for nascent immune complexes was not immediate (60–90 sec) as for pre-formed immune complexes (Schifferli *et al.*, 1988; 1989) or for nascent DNA–anti-DNA complexes formed in rabbits and monkeys (Edberg *et al.*, 1987). The delay in the immune adherence reaction was certainly not due to insufficient complement or C3b receptors which were both present in excess. It is likely that after the initial rapid antigen–antibody interaction, some rearrangements in the lattice of the complex have to take place to allow optimal complement fixation.

The immune adherence reaction occurred in immunized humans only in the presence of a large antibody excess, although immune complexes had formed in all individuals with a detectable level of specific antibodies. Whether this might be the case for other types of immune complexes remains to be explored, but this observation may be relevant to diseases where immune complexes form in large antibody excess such as bacterial endocarditis and shunt nephritis (Levy & Hong, 1973; Kauffmann *et al.*, 1981). We are aware that the fraction of immune complexes bound to erythrocytes was small; however, the immune complexes do not remain attached to erythrocytes *in vivo*; there is a continuous exchange between bound and unbound immune complexes (Schifferli *et al.*, 1988; Ng, Schifferli & Walport, 1988). Thus it is possible that the immune adherence reaction involved more immune complexes than the small fraction detected at any given time. In addition, the immune complexes which were transported by erythrocytes in the circulation were probably those which would have been most likely to induce tissue damage because of their size and complement activating properties.

In vitro, the percentage immune adherence correlated with CR1 number per erythrocytes. Not enough studies have been performed in immunized humans to see whether CR1 is also a limiting factor *in vivo* as shown for preformed immune complexes (Schifferli *et al.*, 1989). Such an analysis is further complicated by the fact that each subject has antibodies with different characteristics. However, it was interesting to see that in the two subjects with similar antibody levels and immune complexes elimination rates but different CR1 number per erythrocytes, immune adherence was higher in the subject with more CR1 per erythrocytes. Further studies are needed in order to confirm this isolated observation.

We have shown that, in humans, immune adherence participates in the elimination of nascent HBsAg/Ab immune complexes. To what extent immune adherence will prevent the localization of immune complexes in tissues outside the fixed macrophage system remains to be determined.

ACKNOWLEDGMENTS

We thank Dr P. Adamovicz for providing the HBsAg, and Dr N. Hogg for providing the mouse anti-CR1 monoclonal Ab E11. We also thank Dr K. Davies for his helpful comments and Miss Gertraud Steiger for

excellent technical assistance. This work was supported by grants from the Fonds National Suisse de la Recherche Scientifique (32/25606.88) and the Swiss Federal Department of Public Health. N. M is supported by the Roche Foundation and the Carlos and Elsie de Reuter Charitable Trust. J.A. S. is a recipient of a Max Cloëtta Career Development Award.

REFERENCES

Bolton, A.E. & Hunter, W.M. (1973) The labelling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent. *Biochem. J.* **133,** 529.

Cornacoff, J.B., Hebert, L.A., Smead, W.L., VanAman, M.E., Birmingham, D.J. & Waxmann, F.J. (1983) Primate erythrocyte immune complex-clearing mechanisms. *J. clin. Invest.* **71,** 236.

Edberg, J.C., Kujala, G.A. & Taylor, R.P. (1987) Rapid immune adherence reactivity of nascent, soluble antibody/DNA immune complexes in the circulation. *J. Immunol.* **139,** 1240.

Fraker, P.J. & Speck, J.C. (1978) Protein and cell iodinations with a sparingly soluble chloramine, 1, 3, 4, 6-tetrachloro-3a, 6a-diphenylglycoluril. *Biochem. Biophys. Res. Commun.* **80,** 849.

Gocke, D.J., Hsu, K., Morgan, C., Bombardieri, S., Lockshin, M. & Christian, C.L. (1970) Association between polyarteritis and Australia antigen. *Lancet,* **ii,** 1149.

Hirschel, B.J., Benusiglio, L.N., Favre, H., Chatelanat, F., Humair, L., Zubler, R.H. & Cruchaud, A. (1977) Glomerulonephritis associated with hepatitis B. Report of a case and review of the literature. *Clin. Nephrol.* **8,** 404.

Kauffmann, R.H., Thompson, J., Valentijn, R.M., Daha, M.R. & Van Es, L.A. (1981) The clinical implications and the pathogenic significance of circulating immune complexes in infective endocarditis. *Am. J. Med.* **71,** 17.

Levy, R.L. & Hong, R. (1973) The immune nature of subacute bacterial endocarditis (S.B.E.) nephritis. *Am. J. Med.* **54,** 645.

Ng, Y.C., Schifferli, J.A. & Walport, M.J. (1988) Immune complexes and erythrocyte CR1 (complement receptor type 1): effect of CR1 numbers on binding and release reactions. *Clin. exp. Immunol.* **71,** 481.

Paccaud, J.P., Steiger, G., Sjöholm, A.G., Spaeth, P.J. & Schifferli, J.A. (1987) Tetanus toxoid-anti-tetanus toxoid complexes: a potential model to study the complement transport system for immune complex in humans. *Clin. exp. Immunol.* **69,** 468.

Paccaud, J.P., Steiger, G. & Schifferli, J.A. (1989) Reduced immune adherence of antigen/antibody complexes formed in the presence of complement. *Complement inflamm.* (In press).

Ross, G.D., Yount, W.J., Walport, M.J., Parker, C.J., Fuller, C.R., Taylor, R.P, Myones, B.J. & Lachmann, P.J. (1985) Disease-associated loss of erythrocyte complement receptors (CR1, C3b receptors) in patients with systemic lupus erythematosus and other diseases involving autoantibodies and/or complement activation. *J. Immunol.* **135,** 2005.

Schifferli, J.A. & Peters, D.K. (1983) Immune adherence and staphylococcus protein A binding of immune complexes produced by complement activation. *Clin. exp. Immunol.* **54,** 827.

Schifferli, J.A., Steiger, G., Hauptmann, G., Spaeth, P. & Sjöholm, A.G. (1985) Formation of soluble immune complexes by complement in sera of patients with various hypocomplementemic states. *J. clin. Invest.* **76,** 2127.

Schifferli J.A., Ng, Y.C., Estreicher, J. & Walport, M.J. (1988) The clearance of tetanus toxoid/anti-tetanus toxoid immune complexes from the circulation of humans: complement- and erythrocyte CR1-dependent mechanisms. *J. Immunol.* **140,** 899.

Schifferli, J.A., Ng, Y.C., Paccaud, J.-P. & Walport, M.J. (1989) The role of hpocomplementaemia and low erythrocyte complement receptor type 1 numbers in determining abnormal immune complex clearance in humans. *Clin. exp. Immunol.* **75,** 329.

Taylor, R.P., Kujala, G., Wilson, K., Wright, E. & Harbin, A. (1985) *In vivo* and *in vitro* studies of the binding of antibody/dsDNA immune complexes to rabbit and guinea-pig platelets. *J. Immunol.* **134,** 2250.

Trepo, C.G., Zuckerman, A.J., Bird, R.C. & Price, A.M. (1974) The role of circulating hepatitis B antigen/antibody imune complexes in the pathogenesis of vascular and hepatic manifestations of polyarteritis nodosa. *J. clin. Pathol.* **27,** 863.

Varga, L., Thiry, E. & Füst, G. (1988) BSA–anti-BSA immune complexes formed in the presence of human complement do not bind autologous red blood cells. *Immunology,* **64,** 381.

Waxman, F.J., Hebert, L.A., Cornacoff, J.B., VanAman, M.E., Smead, W.L., Kraut, E.H, Birmingham, D.J. & Taguiam J.M. (1984) Complement depletion accelerates the clearance of immune complexes from the circulation of primates. *J. clin. Invest.* **74,** 1329.

Waxman, F.J., Hebert, L.A., Cosio, F.G., Smead, W.L, VanAman, W.L, Taguiam, J.M. & Birmingham, D.J. (1986) Differential binding of immunoglobulin A and immunoglobulin G1 immune complexes to primate erythrocytes *in vivo*: immunoglobulin A immune complexes bind less well to erythrocytes and are preferentially deposited in glomeruli. *J. clin. Invest.* **77,** 82.