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Effect of preformed immune complexes on the clearance and tissue localization of single-stranded DNA in mice

W. EMLEN* & M. MANNIK *Department of Medicine, Division of Rheumatology, University of Washington, Seattle, Washington, USA*

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SUMMARY

Recent studies have shown that DNA is cleared from the circulation extremely rapidly by the liver, and that normal individuals have low or immeasurable levels of circulating DNA. In some patients with SLE and in NZB/W mice, however, significant amounts of free DNA as well as DNA–anti-DNA immune complexes have been found in the circulation, suggesting a possible defect in DNA clearance in these conditions. To delineate factors which might contribute to the persistence of DNA in the circulation, we have assessed the effects of immune complexes on the clearance of single stranded DNA in normal C57Bl/6J mice.

HSA–anti-HSA immune complexes at five-fold antigen excess were injected intravenously and after a variable interval, the clearance of single-stranded DNA was determined. Clearance of all doses of DNA was markedly suppressed 6 to 12 hr after the administration of immune complexes and returned to normal by 24 hr. Immune complexes decreased DNA clearance by blocking the hepatic uptake of DNA without altering the distribution of DNA to other organs. Histology and studies on the effect of immune complexes on the clearance of bromosulphophthalein (BSP) and sulphur colloid suggest that immune complexes affect DNA clearance by altering hepatic blood flow.

The results obtained in this study suggest that circulating immune complexes in patients with SLE or in other conditions may suppress normal DNA clearance, and thereby contribute to the persistence of DNA in the circulation.

INTRODUCTION

DNA–anti-DNA immune complexes are thought to play a major role in the pathogenesis of tissue injury in systemic lupus erythematosus (SLE) (Winfield, Koffler & Kunkel, 1975). The presence of DNA antibodies in the circulation is the hallmark of this disease (Koffler *et al.*, 1971), and DNA antibody activity has been demonstrated in SLE tissue eluates (Krishnan & Kaplan, 1967; Koffler, Schur & Kunkel, 1968). Antibodies to DNA in the circulation are not sufficient to induce disease in experimental animals, however, until a source of antigen is provided. Natali & Tan (1972) immunized rabbits with ultraviolet light-treated DNA (u.v.-DNA) and showed that immune complexes and glomerulonephritis were produced only after the appropriate antigen, in this case u.v.-DNA, was made available to combine with circulating antibodies. Lambert & Dixon (1968) showed that immune complex disease in NZB/W mice is greatly accelerated by the injection of additional antigen (DNA). It is pertinent, therefore, to look at the clearance of DNA itself, and at factors which might alter its clearance.

* Present address and address for correspondence: Woodruff Emlen, MD, USPHS Hospital, PO Box 3145, Seattle, Washington 98114, USA.

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Previous work in experimental animals showed that exogenously administered DNA is cleared extremely rapidly (Chused, Steinberg & Talal, 1972; Dorsch *et al.*, 1975) and that this rapid clearance was mediated almost entirely by the liver (Emlen & Mannik, 1978). Consistent with this rapid clearance is the observation that normal persons have very low or immeasurable levels of DNA in the circulation (Steinman, 1975). However, significant levels of DNA have been reported in the circulation of some patients with SLE (Koffler *et al.*, 1973), in patients after trauma or haemodialysis (Steinman & Ackad, 1977), and in female NZB/W mice (Lambert & Dixon, 1968). The presence of DNA in the circulation in these conditions leads one to postulate that large amounts of DNA are released into the circulation or that removal of circulating DNA is slowed. We have previously shown that the clearance mechanism of single-stranded DNA (ssDNA) is saturable (Emlen & Mannik, 1978), so that the release of a large bolus of DNA into the circulation, as might be seen with tissue trauma or after ultraviolet light exposure, could result in a prolonged half-life of circulating DNA. Alterations in the DNA removal mechanism itself could be either (1) intrinsic as a result of genetic differences, or (2) extrinsic as a result of circulating substances which compete with or alter the hepatic removal mechanism.

One group of substances which could potentially alter the hepatic DNA removal mechanism is that of the immune complexes. Immune complexes are found in large quantities in the circulation in SLE (Theofilopoulos, Wilson & Dixon, 1976; Cano *et al.*, 1977), are cleared by the liver (Haakenstad & Mannik, 1974), and as such provide a potential 'extrinsic' factor which might alter the normal DNA removal mechanism. In this study we have examined the effects of preformed immune complexes on the clearance of ssDNA in experimental animals. Our results show that immune complexes do significantly suppress ssDNA clearance and may therefore contribute to the persistence of DNA in the circulation in SLE.

MATERIALS AND METHODS

Preparation of immune complexes. Immune complexes consisting of monomeric human serum albumin (HSA) and anti-HSA were prepared at five-fold antigen excess as described previously in detail (Haakenstad & Mannik, 1974). Preparations were kept in borate buffer (0·2 M sodium borate, 0·15 M NaCl, pH 8·0) at 4°C and spun at 1,000 *g* for 20 min immediately before injection to remove large aggregates. Analysis of these complexes in a linear 10–30% sucrose density gradient revealed a sedimentation pattern identical to the material used in previous studies of the clearance and tissue distribution of immune complexes (Haakenstad & Mannik, 1974).

Preparation of DNA. Calf thymus DNA (Worthington Biochemical, Freehold, New Jersey) was heat-denatured, passed over hydroxyapatite (Biorad, Richmond, California), radioiodinated according to the method of Commerford (1971), and gel-filtered over Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, New Jersey) as previously described (Emlen & Mannik, 1978). This preparation was >90% single-stranded by *Neurospora crassa* endonuclease assay, and had a molecular weight of 150,000–200,000, as determined by polyacrylamide gel electrophoresis (Emlen & Mannik, 1978). All preparations were dialysed against borate buffer (as above) and used in subsequent animal studies.

Preparation of sulphur colloid and BSP. Technitium-labelled sulphur colloid was prepared according to the method of Larson & Nelp (1966), yielding a mean colloid particle size of 0·5–1·0 μm. More than 90% of this material localized in the mononuclear phagocyte system (MPS), primarily the liver, within 15 min (Larson & Nelp, 1966). Bromosulphophthalein (BSP) was diluted with normal saline to the desired dose prior to use.

Animal experiments. Three- to four-month-old female C57Bl/6J mice with no DNA antibody activity were placed on iodized water at least 12 hr prior to experiments. At time zero, animals were injected with immune complexes containing 0·5, 2·0 or 5·0 mg of antibody in 0·35 ml borate buffer, or 0·35 ml borate buffer alone (controls). One, 3, 6, 12, 24 or 48 hr after the administration of immune complexes or borate buffer, animals were given a test dose of either ^{125}I-ssDNA, ^{99m}Tc sulphur colloid or BSP, and clearance of these substances was determined as described below.

Test doses of ^{125}I-ssDNA (10, 50 and 100 μg) were injected into the tail vein in 0·5 ml borate and

20-μl blood samples were obtained from the retro-orbital venous plexus at 0·5, 1, 2, 3, 5, 10, 20 and 30 min. Blood samples were washed immediately from micropipettes with 0·75 ml borate buffer into 0·25 ml carrier DNA solution containing 250 μg of unlabelled DNA, and 1·0 ml cold 10% trichloroacetic acid was added. The samples were centrifuged at 2,500 *g* for 20 min at 4°C, the supernatant removed, and the precipitate and supernatant counted separately in an automatic, well-type gamma counter (Searle, Chicago, Illinois). Injections of 0·25 ml ^{99m}Tc sulphur colloid or 200 μg BSP in 0·25 ml were made into the tail vein and blood samples obtained as above. The disappearance of sulphur colloid was determined by counting radioactivity in 20-μl whole blood samples diluted with 0·75 ml borate. BSP determinations were done according to the method of Seligson, Marino & Dodson (1957).

Specific organ uptake of ^{125}I-ssDNA was determined as described previously (Haakenstad, Case & Mannik, 1975) using ^{131}I-labelled monomeric rabbit IgG as a plasma volume marker.

Analysis of data. Immediately prior to each experiment, a sample of the ssDNA to be injected was counted and a specific activity in c.p.m./μg was determined. Using this specific activity, counts remaining in the 10% TCA precipitate were converted to μg ssDNA remaining/ml, and the concentration versus time data were plotted on semilogarithmic paper. Plasma BSP concentrations were determined from absorbance readings using a standard curve and plotted as above. Since it is difficult to determine the concentration of colloidal particles in ^{99m}Tc sulphur colloid, results were expressed as c.p.m./20-μl sample. To avoid variations in particle concentration, control and experimental groups were tested with a fixed dose of the same preparation of ^{99m}Tc sulphur colloid. A ^{99m}Tc standard was counted just prior to counting samples from each mouse, and corrections were made at each time point for decay of ^{99m}Tc.

Clearance velocities for all three test substances were calculated using the formula $V = 2{\cdot}3026\ KS$, where S and K are the y intercept and slope, respectively, of the lines obtained by linear regression analysis of the early data points. Only those data points which graphically fell in the linear portion of the disappearance curves were used for calculation of K and S. Half-lives were calculated according to the formula $t\ 1/2 = 0{\cdot}693/2{\cdot}3026\ K$. The clearance velocity and half-life was calculated for each individual animal, and comparisons between the borate and immune complex-pretreated groups were made using Student's *t*-test.

Histology. Three, 6, 12, 24 and 72 hr after the administration of immune complexes containing 2·0 and 5·0 mg of antibody, animals were killed, their livers removed and fixed in buffered formalin. One-micron sections were stained with haematoxyin and eosin and examined by three observers who did not know the identity of the specimens. Sections were examined with particular attention to hepatic necrosis, inflammatory cell infiltration, and sinusoidal spaces.

RESULTS

Effect of immune complexes on clearance of ssDNA

The administration of immune complexes significantly decreased the clearance rate of a subsequent dose of ssDNA. As shown in Fig. 1, immune complexes containing 5 mg of antibody administered 6 hr before the injection of 50 μg of ssDNA decreased the DNA clearance velocity by almost 50%, from 2·7 to 1·4 $\mu g \cdot ml^{-1} \cdot min^{-1}$. The half-life of this dosage of DNA was increased from 6·5 to 12·5 min. Doses of immune complexes containing 0·5 and 2·0 mg of antibody also decreased DNA clearance velocity but to a lesser degree.

The clearance of 10, 50 and 100 μg of ssDNA was studied 6 hr after a borate or immune complex (5 mg antibody) preload. DNA clearance was saturable with large doses of DNA, as we have shown previously (Fig. 2). The immune complex-pretreated animals showed decreased DNA clearance at all doses of administered DNA, resulting in a downward shift of the entire curve. As is apparent from Fig. 2, immune complex-induced differences in DNA clearance were more easily demonstrable with larger doses of DNA. The maximum clearance velocity (Vmax) and Km (Michaelis–Menten constant) of DNA clearance in borate and in immune complex-preloaded animals were calculated from reciprocal Lineweaver–Burke plots of the data. Vmax was decreased from 4·25 to 2·33 $\mu g \cdot ml^{-1} \cdot min^{-1}$ by immune complexes, but the Km was not changed.

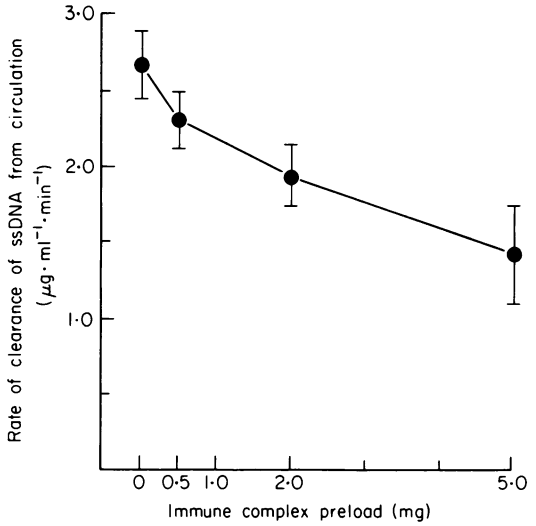

Fig. 1. Rate of clearance ($\mu g \cdot ml^{-1} \cdot min^{-1}$) of a 50-$\mu$g dose of ssDNA injected 6 hr after the administration of immune complexes or borate (controls). Each point represents the mean ± s.d. of at least six mice. Immune complexes containing 2·0 mg and 5·0 mg of antibody significantly decreased ssDNA clearance ($P < 0{\cdot}001$); the effect of 0·5 mg of immune complexes was of borderline significance ($0{\cdot}05 < P < 0{\cdot}1$).

Effect of the interval after administration of immune complexes on ssDNA clearance

To examine the effect of immune complexes on the clearance of ssDNA over time, immune complexes containing 5·0 mg of antibody or borate were administered at time zero. One, 3, 6, 12, 24 and 48 hr later the clearance of a 50-μg dose of ssDNA was determined. The administration of immune complexes caused an almost immediate increase in vascular permeability with resultant haemoconcentration and decreased intravascular volume (Haakenstad, Case & Mannik, 1975). One hour after the administration of complexes, however, the haematocrit and overall appearance of the mice had returned to normal. To exclude intravascular volume differences which might affect DNA clearance, the volume of distribution of DNA was calculated for each animal. No differences between the borate and immune complex-pretreated animals were detected, indicating that intra-

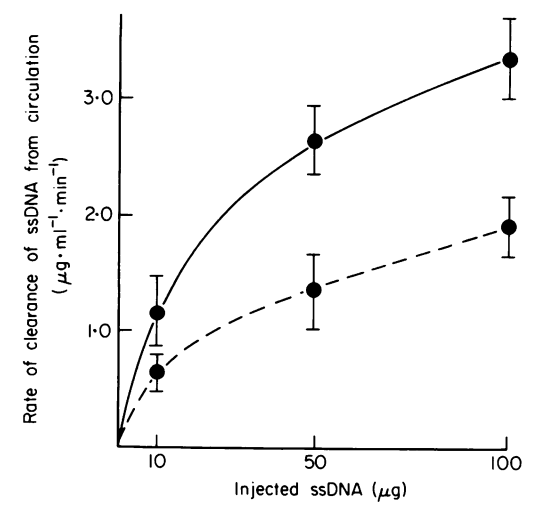

Fig. 2. Rate of clearance of varying doses of ssDNA 6 hr after the administration of borate (controls; •——•) or immune complexes (•– – –•) containing 5·0 mg of antibody. Each point represents the mean ± s.d. for six mice. Immune complexes decrease the clearance of all doses of DNA.

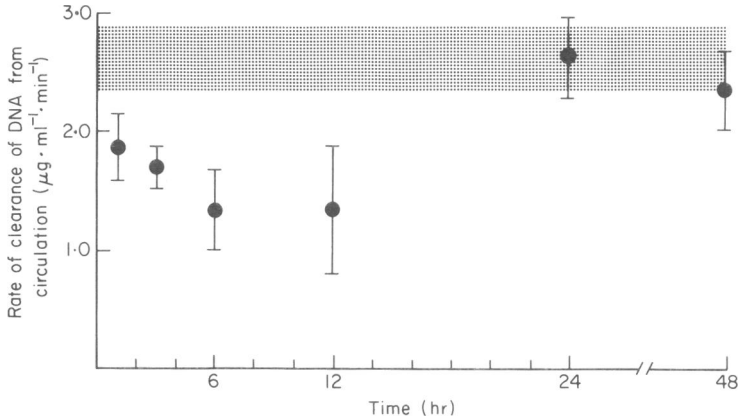

Fig. 3. Rate of clearance of 50 μg ssDNA at varying intervals after the administration of immune complexes containing 5·0 mg of antibody. Each point represents the mean ± s.d. of at least four mice. The stippled area represents the mean clearance rate ± 1 s.d. for animals pretreated with borate ($n = 12$). DNA clearance was significantly decreased 1 hr ($P < 0.05$), 3 hr ($P < 0.01$), 6 hr ($P < 0.001$) and 12 hr ($P < 0.01$) after the administration of complexes, but had returned to normal by 24 and 48 hr.

vascular volume had returned to normal in the immune complex-treated mice by the time the first test dose of DNA was administered (1 hr).

ssDNA clearance was depressed maximally 6 and 12 hours after the administration of complexes; moderately, although significantly, at 1 and 3 hr, and had returned to normal by 24 hr (Fig. 3). Previous work (Haakenstad & Mannik, 1976) has shown that immune complexes persist in the liver for up to 24 hr after the administration of a dose of immune complexes containing 5·0 mg of antibody. Fig. 4 shows the per cent decrease in clearance velocity of ssDNA and the amount of immune complexes in the liver at varying times, as determined by Haakenstad & Mannik (1976), after a dose of immune complexes containing 5·0 mg of antibody. It is evident that the degree of inhibition of ssDNA clearance correlates closely with the amount of immune complexes present in the liver.

Effect of immune complexes on organ uptake

Previous studies have shown that hepatic uptake accounts for more than 90% of ssDNA removed

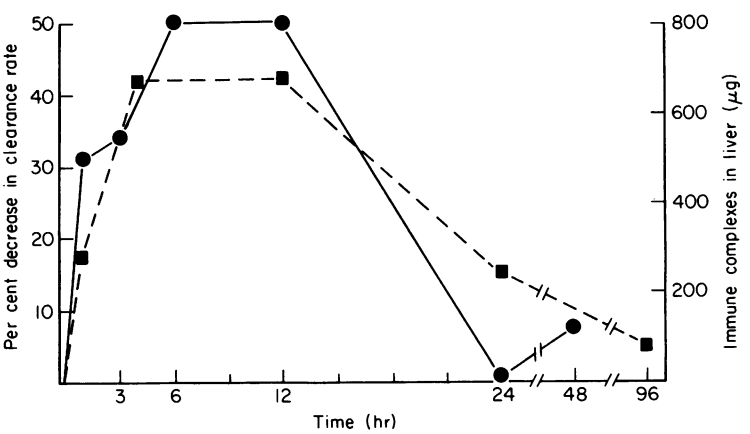

Fig. 4. Relationship between suppression of DNA clearance (●——●) and the amount of immune complexes present in the liver (■– – –■) at varying times after the administration of immune complexes containing 5·0 mg of antibody. The degree of inhibition of DNA clearance closely parallels the amount of immune complexes in the liver.

Table 1. Specific organ uptake of ssDNA

	Buffer pretreated*	Immune complex pretreated*
Liver†	15·10 ± 1·10‡	7·59 ± 2·26
Spleen	0·70 ± 0·23	0·51 ± 0·43
Kidneys	0·15 ± 0·05	0·17 ± 0·15

* Mice were preloaded with borate buffer or immune complexes containing 5·0 mg of antibody 6 hr before the administration of DNA.

† Organs removed 3 min after injection of 50 μg ssDNA.

‡ Mean uptake (μg) ± 1 s.d. of four mice; $P < 0{\cdot}01$ for liver uptake.

from the circulation (Emlen & Mannik, 1978). We therefore measured specific organ uptake of ssDNA in animals preloaded 6 hr earlier with borate or with immune complexes containing 5·0 mg of antibody. In order to minimize the effects of hepatic storage or reuptake of DNA breakdown products, organs were removed 3 min after the administration of a 50-μg dose of ssDNA and specific organ uptake was calculated. Total hepatic uptake was markedly decreased in immune complex-pretreated mice (Table 1), but spleen and kidney uptake were not significantly altered. Thus, the inhibition of ssDNA clearance by immune complexes is caused by decreased hepatic uptake of DNA.

Effects of immune complexes on BSP and sulphur colloid clearance

To examine the specificity of the immune complex blockade of ssDNA clearance, the clearance of BSP and sulphur colloid was studied 6 hr after the administration of immune complexes (5·0 mg of antibody). The half-life of a 200-μg test dose of BSP was almost doubled from 0·92 min in borate-pretreated animals to 1·67 min in immune complex-pretreated mice. Sulphur colloid clearance was similarly decreased, with prolongation of the sulphur colloid half-life from 1·48 to 3·26 min. The effects of immune complexes on the clearance of both BSP and sulphur colloid were statistically significant to the $P < 0{\cdot}002$ level. Immune complexes decreased DNA clearance by 50·2%, BSP clearance by 45% and sulphur colloid clearance by 48%.

Effect of immune complexes on liver histology

Three, 6, 12, 24 and 72 hr after the administration of immune complexes containing 2·0 or 5·0 mg of antibody, liver tissue was obtained and examined blindly by three observers. Liver histology was normal at 3, 24 and 72 hr. Six and 12 hr after the administration of immune complexes, small foci of polymorphonuclear leucocytes and some obliteration of sinusoidal spaces were seen. This was most marked with the 5-mg dose, but was noted to a lesser degree by two observers with the 2-mg dose as well. At no time was there any frank hepatic necrosis or gross architectural change.

DISCUSSION

The effect of immune complexes on DNA clearance is pertinent to our understanding of SLE because of the large quantitites of immune complexes which have been demonstrated in the circulation of SLE patients (Theofilopoulos *et al.*, 1976; Cano *et al.*, 1977). Immune complexes are cleared by the mononuclear phagocyte system (MPS) of the liver, and in large doses have been shown to saturate the MPS (Haakenstad & Mannik, 1974). As such, immune complexes might be able to induce a form of MPS blockade, and disrupt the clearance of other substances (Normann, 1974). Decreased clearance of antigens such as DNA could lead to their persistence in the circulation, thereby providing additional antigen for the production of immune complexes, or for contact with immunologically active cells.

This study has examined the effects of preformed HSA–anti-HSA immune complexes on the clearance of ssDNA in normal mice with no DNA antibody activity. Our data show that immune complexes decrease the clearance of DNA from the circulation. The degree of suppression of DNA clearance is proportional to the dose of immune complexes, with maximal suppression occurring after a saturating dose of complexes (Fig. 1). However, DNA clearance is slowed after as little as 0·5 mg of immune complexes. In a mouse with a 2-ml blood volume, 0·5 mg of complexes corresponds to an immune complex concentration of 250 μg/ml. Theofilopoulos *et al.* (1976), using the Raji cell assay, have reported levels of immune complexes as high as 1,000 μg/ml in the sera of some SLE patients. Direct comparisons between immune complex levels in humans determined by the Raji cell assay and the calculated levels in our mice are obviously not possible. However, such data do indicate that the immune complex levels used in our study are within the same order of magnitude as may be attained under physiological conditions in some diseases.

Studies on the effects of immune complexes on MPS function in human SLE have recently been reported by Frank *et al.* (1979). They have shown that the clearance of IgG-coated red cells is decreased in active SLE, and that the magnitude of this decrease is proportional to the level of circulating immune complexes. Sensitized red cells are cleared primarily by the splenic MPS (Atkinson, Schreiber & Frank, 1973), however, and therefore the relevance of these findings for hepatic MPS function or for hepatic clearance of DNA remains unclear. Further studies of hepatic MPS function in SLE are necessary to define the effects of immune complexes on the clearance of DNA in SLE.

In addition to demonstrating immune complex blockade of DNA clearance, our data provide some insight into the mechanism of this blockade. Under normal conditions greater than 90% of an administered dose of ssDNA is removed from the circulation by binding to the liver (Emlen & Mannik, 1978). Our data (Table 1) indicate that immune complexes slow ssDNA clearance by inhibiting its uptake by the liver. Furthermore, the degree of DNA clearance suppression at any given time after the administration of immune complexes closely parallels the amount of complexes present in the liver at that time (Fig. 4). Thus, the presence of immune complexes in the liver in some way alters the liver's ability to bind DNA.

As shown in Fig. 3, there is a delay in the effect of immune complexes on DNA clearance, reaching a maximum at 6 and 12 hr. Shortly after immune complex injection, when circulating immune complex levels are maximum, the effect on DNA clearance is minimal. By 6 hours, large-latticed immune complexes are almost completely removed from the circulation, but their effect on DNA clearance is reaching a maximum. This delay in suppression of DNA clearance strongly suggests that circulating immune complexes do not act by competitive inhibition of DNA uptake by the liver. This is further substantiated by the DNA dose/clearance velocity curves (Fig. 2), which show that immune complexes alter the Vmax of DNA clearance, but not the Km. Applying Michaelis–Menten enzyme kinetics analysis to the MPS, as has been done by Normann (1974), this pattern of inhibition indicates non-competitive inhibition, as might be seen with a decreased number of available binding sites for DNA.

Immune complexes could alter hepatic binding of DNA in several ways: (1) uptake of large amounts of complexes via membrane receptors might deplete Kupffer cells or hepatocytes of receptors necessary for DNA binding; (2) immune complexes might be directly toxic to liver cells, or (3) complexes might induce alterations in liver blood flow and thereby alter DNA clearance. Immune complexes bind to Kupffer cells primarily via Fc receptors (Haakenstad & Mannik, 1976), and while it is unlikely that DNA binds to Fc receptors, adjacent membrane receptors which might be involved in DNA binding could be depleted as membrane is interiorized with the phagocytosed immune complexes. However, Schmidt & Douglas (1972) have shown that macrophage receptors are re-expressed within 2–6 hr after saturation with immune complexes. The prolonged time course of decreased hepatic DNA binding which we have observed therefore makes this explanation unlikely. However, direct testing using isolated cell systems is in progress to explore this question further.

Direct hepatic toxicity of immune complexes has been demonstrated by Steiner (1961). He induced hepatitis and irreversible hepatic necrosis by the direct injection of immune complexes into the portal or systemic circulation of the rabbit. To examine the possibility that the changes in DNA

clearance which we observed might be due to hepatic necrosis, the livers of control and immune complex-pretreated mice were examined by light microscopy. Histological changes consisting of small foci of polymorphonuclear leucocytes and some decrease in sinusoidal spaces were seen at 6 and 12 hr after the administration of complexes. Histology was normal at 3, 24 and 72 hr. Thus, histologic changes were closely parallel to the functional blockade of DNA clearance.

If DNA clearance suppression were related to immune complex-induced changes in liver histology, we postulated that this suppression should be a non-specific phenomenon. We therefore tested the effects of immune complexes on the clearance of BSP and ^{99m}Tc sulphur colloid (TSC). BSP is cleared by hepatocytes (van Bezooijen, Grell & Knook, 1976), whereas TSC is cleared by Kupffer cells (George *et al.*, 1978). The clearance of these two dissimilar substances is suppressed equally by an equal dose of immune complexes, and the degree of this suppression is almost identical to that seen with DNA. We conclude that in the model we have used, immune complexes transiently induce foci of inflammation or cellular swelling in the liver which non-specifically alter the clearance of DNA, BSP and TSC. Direct hepatocellular toxicity of immune complexes is a possible mechanism for the suppressed clearance, but the relative paucity of histological changes makes this hypothesis unattractive. It is known that the clearance of substances with a short half-life or high extraction ratio, such as DNA, BSP and TSC, is extremely sensitive to hepatic blood flow (Shand, 1977). Foci of inflammation or Kupffer cell distention with phagocytosed immune complexes could result in intrahepatic shunting of blood flow as has been proposed recently by Wood *et al.* (1979), with a subsequent decrease in the efficiency of hepatic extraction.

The mechanism of immune complex suppression of DNA clearance, and the significance of this observation for SLE or other immune complex diseases are not yet clear. Our model uses a single injection of a high dose of immune complexes rather than the more physiological setting of continuous formation of small amounts of immune complexes. Further studies using animal models of SLE will be necessary before the relevance of our findings can be defined. It is clear, however, that under some conditions immune complexes alter the hepatic clearance of ssDNA and potentially a number of other substances. In SLE, this phenomenon may contribute to the observed persistence of DNA in the circulation, thereby providing additional antigen for the formation of immune complexes.

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REFERENCES

ATKINSON, J.P., SCHREIBER, A.S. & FRANK, M.M. (1973) Effects of corticosteroids and splenectomy on the immune clearance and destruction of erythrocytes. *J. clin. Invest.* **52,** 1509.

CANO, P.O., JERRY, L.M., SLADOWSKI, J.P. & OSTERLAND, C.K. (1977) Circulating immune complexes in systemic lupus erythematosus. *Clin. exp. Immunol.* **29,** 197.

CHUSED, T.M., STEINBERG, A.D. & TALAL, N. (1972) The clearance and localization of nucleic acids by New Zealand and normal mice. *Clin. exp. Immunol.* **12,** 465.

COMMERFORD, S.L. (1971) Iodination of nucleic acids *in vitro*. *Biochemistry*, **10,** 1993.

DORSCH, C.A., CHIA, D., LEVY, L. & BARNETT, E.V. (1975) Persistence of DNA in the circulation of immunized rabbits. *J. Rheumatol.* **212,** 161.

EMLEN, W. & MANNIK, M. (1978) Kinetics and mechanisms for removal of circulating single-stranded DNA in mice. *J. exp. Med.* **147,** 684.

FRANK, M.M., HAMBURGER, M.I., LAWLEY, T.J., KIMBERLY, R.P. & PLOTZ, P.H. (1979) Defective Fc-receptor function in lupus erythematosus. *N. Engl. J. Med.* **300,** 518.

GEORGE, E.A., HENRY, R.E., FLETCHER, J.W. & DONATI, R.M. (1978) Mechanisms of hepatic extraction of gelatin stabilized Tc-99m sulfur colloid (TSC). *J. nucl. Med.* **19,** 681 (abstract).

HAAKENSTAD, A.O., CASE, J.B. & MANNIK, M. (1975) Effect of cortisone on the disappearance kinetics and tissue localization of soluble immune complexes. *J. Immunol.* **114,** 1153.

HAAKENSTAD, A.O. & MANNIK, M. (1974) Saturation of the reticuloendothelial system with soluble immune complexes. *J. Immunol.* **112,** 1939.

HAAKENSTAD, A.O. & MANNIK, M. (1976) The disappearance kinetics of soluble immune complexes prepared with reduced and alkylated antibodies and with intact antibodies in mice. *Lab. Invest.* **35,** 283.

KOFFLER, D., AGNELLO, V., WINCHESTER, R. & KUNKEL, H.G. (1973) The occurrence of single stranded

DNA in the serum of patients with systemic lupus erythematosus and other diseases. *J. clin. Invest.* **52,** 198.

KOFFLER D., CARR, R., AGNELLO, V., THOBURN, R. & KUNKEL, H.G. (1971) Antibodies to polynucleotides in human sera: antigenic specificity and relation to disease. *J. exp. Med.* **134,** 294.

KOFFLER, D., SCHUR, P.H. & KUNKEL H.G. (1968) Immunological studies concerning the nephritis of systemic lupus erythematosus. *J. exp. Med.* **126,** 607.

KRISHNAN, C. & KAPLAN, M.H. (1967) Immunopathologic studies of systemic lupus erythematosus. II. Antinuclear reaction of γ-globulin eluted from homogenates and isolated glomeruli of kidneys from patients with lupus nephritis. *J. clin. Invest.* **46,** 569.

LAMBERT, P.H. & DIXON, F.J. (1968) Pathogenesis of the glomerulonephritis of NZB/W mice. *J. exp. Med.* **125,** 507.

LARSON, S.M. & NELP, W.B. (1966) Radiopharmacology of a simplified technetium-99m colloid preparation for photoscanning. *J. nucl. Med.* **7,** 817.

NATALI, P.G. & TAN E.M. (1972) Experimental renal disease induced by DNA–anti-DNA immune complexes. *J. clin. Invest.* **51,** 345.

NORMANN, S.J. (1974) Kinetics of phagocytosis. II. Analysis of *in vivo* clearance with demonstration of competitive inhibition between similar and dissimilar foreign particles. *Lab. Invest.* **31,** 161.

SCHMIDT, M.E. & DOUGLAS, S.D. (1972) Disappearance and recovery of human monocyte IgG receptor activity after phagocytosis. *J. Immunol.* **109,** 914.

SELIGSON, D., MARINO, J. & DODSON, E. (1957) Determination of sulfobromophthalein in serum. *Clin Chem.* **3,** 638.

SHAND, D. (1977) Drug disposition in liver disease. *N. Engl. J. Med.* **296,** 1527.

STEINER, J.W. (1961) Investigations of allergic liver injury. I. Light, fluorescent and electron microscopic study of the effects of soluble immune aggregates. *Am. J. Pathol.* **38,** 411.

STEINMAN, C.R. (1975) Free DNA in serum and plasma from normal adults. *J. clin. Invest.* **56,** 512.

STEINMAN, C.R. & ACKAD, A. (1977) Appearance of circulating DNA during hemodialysis. *Am. J. Med.* **62,** 693.

THEOFILOPOULOS, A.N., WILSON, C.B. & DIXON, F.J. (1976) The Raji cell radioimmunoassay for detecting immune complexes in human sera. *J. clin. Invest.* **57,** 169.

VAN BEZOOIJEN, C.F., GRELL, T. & KNOOK, D.L. (1976) Bromosulfophthalein uptake by isolated liver parenchymal cells. *Biochem. Biophys. Res. Commun.* **69,** 354.

WINFIELD, J.B., KOFFLER, D. & KUNKEL, H.G. (1975) Role of DNA–anti-DNA complexes in the immunopathogenesis of tissue injury in systemic lupus erythematosus. *Scand. J. Rheumatol.* **11**(Suppl.), 59.

WOOD, A.J., VILLENEUVE, J.P., BRANCH, R.A., ROGERS, L.W. & SHAND, D.G. (1979) Intact hepatocyte theory of impaired drug metabolism in experimental cirrhosis in the rat. *Gastroenterology,* **76,** 1358.