

Immunoglobulin Class and Subclass Distribution of Dextran-Reactive Antibodies in Human Reactors and Non Reactors to Clinical Dextran

D. KRAFT,¹ H. HEDIN,² W. RICHTER,² O. SCHEINER,¹
H. RUMPOLD¹ and MADELEINE E. DEVEY³

¹Institute of General and Experimental Pathology, University of Vienna, Austria; ²Departments of Clinical and Biological Research, Pharmacia AB, Uppsala, Sweden and ³Immunology Unit, London School of Hygiene and Tropical Medicine, U.K.

The red cell-linked antigen-antiglobulin reaction (RCLAAR) with stearyl dextran-coated erythrocytes was used to characterize the immunoglobulin (Ig) classes and IgG subclasses of dextran reactive antibodies (DRA) in 27 dextran reactors (DR) and 96 non reactors (DNR). High titres of dextran reactive IgG were regularly found in sera of patients with severe dextran-induced anaphylactoid/anaphylactic reactions (DIAR) prior to the infusion. In four lethal cases IgG antibodies were found to be in the highest titre range of 16,384 to 32,768. In addition, high IgA and IgM titres were also found in severe DIAR. DNR had much lower titres of dextran reactive antibodies of IgG, IgM and IgA classes and IgD antibody was absent in both groups. Dextran reactive IgE antibodies were not demonstrable in DR. Dextran reactive IgG₂, IgG₃, IgG₄ and IgG₁ (indirect measurement) were demonstrated in both DR and DNR. Dextran infusion caused variable neutralization in all Ig classes and IgG subclasses, but the contribution of IgG₂ was considered most important because of its high titres and most pronounced neutralization in severe DIAR. It is concluded that DRA mainly of the IgG class, play a critical pathogenic role in the induction of severe DIAR, which accordingly is classified as immune complex (Type III) anaphylaxis. The method of RCLAAR allows to delineate a risk group of about 2% of potential reactors.

Key words: antibody assessment; dextran-induced anaphylactoid/anaphylactic reactions; RCLAAR; immunoglobulin classes; IgG-subclasses.

Accepted for publication 11 March 1982

CLINICAL ASPECTS

Predisposition to severe dextran-induced anaphylactoid/anaphylactic reactions (DIAR) resides in a small subpopulation of individuals with high titres of dextran-reactive antibodies. Infusion of clinical dextran into such patients generates immune complexes which obviously produce symptoms of type III anaphylaxis by complement activation and release of mediators. By use of the red cell-linked antigen-antiglobulin reaction (RCLAAR) predominantly IgG-class antibodies could be detected and are considered to play a crucial pathogenic role. In mild DIAR, both antibody-dependent and -independent mechanisms may occur.

Dextran has been used clinically for more than 30 years for plasma volume expansion, improved blood flow, and thromboprophylaxis (19). Like other plasma volume expanders, dextran may elicit rare hypersensitivity reactions (28). These range from skin manifestations to circulatory shock, which is sometimes

fatal, and are classified into severity grades I to IV (Table 1). Studies on the pathomechanisms of dextran-induced anaphylactoid/anaphylactic reactions (DIAR) have shown that natural precipitating dextran reactive antibodies (DRA) are present in patients with grade III and IV reactions (9, 15, 27); no DRA of IgE-class were

found (10). Complement activation via the classical pathway occurred (8) and histopathological changes in patients with fatal DIAR accorded with the picture of experimental immune complex anaphylaxis (29). All these findings indicate that dextran infusion generates harmful immune complexes, which act as elicitors of severe DIAR (Type III allergic reaction). In clinical trials on more than 100,000 patients, the hapten inhibition principle has been applied successfully to prevent severe DIAR, confirming their antibody dependence. Mild DIAR (grades I and II) may be antibody dependent or not (9). For review of the incidence, pathomechanisms and prevention of DIAR, see ref. 25.

Clinical dextran itself does not induce formation of DRA (6), but higher molecular weight dextran fractions may do so (13). Native dextran may be present in dental plaques or be ingested as a contaminant of sucrose (4, 22). It has also recently been shown to occur in traces in sera of normal individuals and in higher levels in patients with gastrointestinal diseases (24). The antigenic determinant of clinically used B 512 dextran, which is made up of three to six α 1-6 linked glucose residues, also occurs in polysaccharides of bacteria such as pneumococci, streptococci, Klebsiella, Salmonella and others (14). This explains why DRA of low or medium titres are widely distributed in the normal human population (10, 12). For routine estimation of DRA, we have previously used a passive haemagglutination assay which measures total activity of all Ig-classes. The purpose of the present study was to determine the individual Ig-classes and IgG-subclasses of DRA in dextran reactors (DR) and non reactors (DNR). A modification of the red cell-linked antigen-antibody reaction (2) using stearoyl as linking agent for dextran was employed.

MATERIAL AND METHODS

Conjugates. Stearoyldextran was prepared from a B512 dextran fraction of MW 70,000 according to the method of Hämmerling & Westphal (7). The degree of substitution corresponded to one stearoyl group per 20 glucose residues.

Antidextran antiserum. Hyperimmune rabbit antidextran antiserum containing 5.2 mg/ml of specific antibody was raised as described earlier by Richter & Kågedal (26) and used for direct haemagglutination against specifically sensitized and unsensitized human red cells of blood group O Rhesus positive (positive and negative controls).

Human sera. Serum samples from 27 patients, who had experienced DIAR, were collected during a 10-year period from Sweden, Norway and Germany. The group consisted of 12 females (mean age 61 years, range 52-79 years) and 15 males (mean age 60 years, range 48-72 years). In all these cases, samples were obtained from the time before the reaction (2 days to 2 min) and in 16 patients samples were also collected 1-8 h after the reaction. The clinical symptoms were classified into severity grades I to IV (Table 1). Four grade IV reactions were fatal. All other patients recovered uneventfully. Patients with grade I, II, and III-IV reactions had received volumes of clinical dextran of 101-500 ml, 25-100 ml, and 1-10 ml respectively before the infusion was discontinued.

Serum samples from 96 patients, who had tolerated infusion of 500 ml of Macrodex®, served as controls. Samples obtained from five hospitals in Sweden were collected prior to the infusion. The group consisted of 50 females (mean age 58 years, range 23-84 years) and 46 males (mean age 62 years, range 17-84 years). The clinical diagnosis indicated that the necessity of dextran treatment was similar for dextran reactors and non reactors.

Table 1
Severity scale for grading of DIAR

Grade	Symptoms
I	Skin manifestations (flush, urticaria)
II	Mild hypotension, tachycardia, nausea, dyspnoea
III	Shock, life-threatening bronchospasm
IV	Cardiac and/or respiratory arrest

(From Ring & Messmer (28)).

Normal human serum (NHS) pool. Equal amounts of serum samples from 21 healthy laboratory workers were pooled and this pool used as reference serum in each test.

Coated erythrocytes. Human red cells of blood group O Rhesus positive were freshly drawn from healthy volunteers just before each test, and washed five times in phosphate buffered saline (PBS). 0.2 ml packed red cells were incubated in 10 ml PBS containing 0.13 mg stearoyldextran. The mixture was kept at 37°C for 30 min. Cells were then spun down, washed three times with PBS and used as a 2% sensitized red cell suspension.

Class- and subclass-specific antiglobulin reagents. Several batches of antisera to human Ig were purchased from Behringwerke, Marburg, FRG, and tested for specificity using red cells coated with myeloma proteins. For this purpose purified IgG, IgM, IgA and IgD myeloma proteins were linked to human red cells of blood group O Rhesus positive by the chromic chloride method of Gold & Fudenberg (5). For the RCLAAR the following antiglobulin reagents (with titres of 1:64 or less with the irrelevant Ig myeloma proteins) were used: anti-IgG, batch No. 2733 D (final dilution 1:400); anti-IgM, batch No. 2716 K (final dilution 1:200); anti-IgA, batch No. 2919 A (final dilution 1:100) and anti-IgD, batch No. 2604 E (final dilution 1:100).

Lyophilized ^{125}I -labelled rabbit antihuman IgE ($\text{D}_{\text{E}2}$) was obtained from Pharmacia AB, Uppsala, as a component of RAST kits. According to the manufacturer, the anti-IgE was purified by immunosorbent techniques and contained 1 $\mu\text{Ci}/\text{ml}$ at the date of labelling. It was reconstituted according to the instructions for use.

The antisera to IgG₂, IgG₃ and IgG₄ were those described by Devey & Voak (3). Anti-IgG₄ was raised in mice by Dr. H. G. Kunkel and anti-IgG₂ and -IgG₃ were raised in baboons using purified myeloma proteins. All the anti-subclass sera were absorbed with myeloma proteins of the unwanted classes, subclasses and light chains coupled to CNBr-activated Sepha-

rose 4B (Pharmacia) on short columns. Specificity was tested by haemagglutination using red blood cells coated by chromic chloride with purified IgG₁, IgG₂, IgG₃, IgG₄, IgG, IgA or IgM myeloma proteins (0.3 mg protein/0.1 ml packed erythrocytes).

To avoid unwanted reactivity the chosen dilutions of the anti-subclass antiglobulins were 2 to 3 dilution steps above the highest cross-reacting dilution and the antiglobulins were used at the following dilutions: anti-IgG₂ 1:40, anti-IgG₃ 1:300, anti-IgG₄ 1:100. For control of reactivity the IgG load on myeloma protein-coated cells was compared to the amount of IgG attached to dextran-coated RBC (using patient sera with titres of $\sim 1:8000$) and the specificity of reactions was also established by inhibition experiments. All antiglobulin reagents were carefully absorbed with dextran-coated human red cells of blood group O Rhesus positive.

Red cell-linked antigen-antiglobulin reaction (RCLAAR). Equal volumes of 2% sensitized human red cells were mixed with serial twofold dilutions of the human serum to be tested. After 30 min at room temperature the cells were washed twice and uptake of specific antibody to the linked antigen was measured with class- and subclass-specific antiglobulin reagents. Suspensions in PBS of dextran-coated human red cells which had not been incubated with human serum, were included as extra controls to ensure that the antiglobulin reagent did not react with sensitized human red cells.

*Red cell-linked antigen-radioactive antiglobulin reaction (RCLA*AR).* 200 μl of 2% sensitized human red cells were incubated with 100 μl of 1:10 diluted serum for 3 h at room temperature. After washing twice with 0.1% gelatine-PBS the cells were resuspended in 600 μl 1% HSA-PBS, and 250 μl were transferred twice to plastic tubes to make duplicates. ^{125}I -antihuman IgE solution (50 μl) was added to each tube and the tubes rotated at an angle of 45° at room temperature for 17 h. After washing 3 times with 0.1% gelatine-PBS, the radioactivity retained by the cells was counted in a γ -counter (17).

Statistics. Results were analysed by the $2 \times 2 \chi^2$ -test, the sign test and the Friedman test.

RESULTS

DRA of IgG, IgM and IgA classes in non reactors and reactors to dextran

Serum samples from 96 patients who showed no adverse reactions upon dextran infusion were collected prior to treatment and antibody titres measured by RCLAAR. The distribution pattern in the Ig classes is shown in Fig. 1. Titres ranged from 2 to 2,048 and the majority were normally distributed. Median titres were as follows: IgG = 16, IgM = 8 and IgA = 4. Of these sera 13.5% were negative for IgG, 26.0% for IgM and 35.4% for IgA antibodies. Serum samples from 27 patients who showed adverse reactions of grades I to IV in connection with dextran infusions had medium to very high levels of DRA of IgG, and also of IgM and IgA classes (Fig. 1). Significantly higher titres were found in reactors in all three Ig classes ($P < 0.01$) as tested by the $2 \times 2 \chi^2$ tests. Median

titres were as follows: IgG = 2,048, IgM = 512 and IgA = 512.

DRA of IgG, IgM and IgA classes in reactors experiencing DIAR of varying severity

IgG titres of patients with DIAR correlated with the severity of clinical symptoms (Fig. 2) and the fatal cases all appeared in the highest range (titres of 16,384 to 32,768). Dextran reactive IgA also showed this correlation but less pronounced with a wider spread of titres in severe DIAR. In the case of IgM, no major titre differences were seen among DIAR of grades II to IV. In grade I reactions, the distribution patterns of low or medium titres were similar in all Ig classes tested.

DRA of IgD and IgE classes in reactors and non reactors to dextran

No evidence for dextran reactive IgD antibodies was obtained in sera of 27 DR and 96 DNR. Dextran reactive IgE antibodies were not observed in any of the sera of the DR by use of the radioactive RCLAAR.

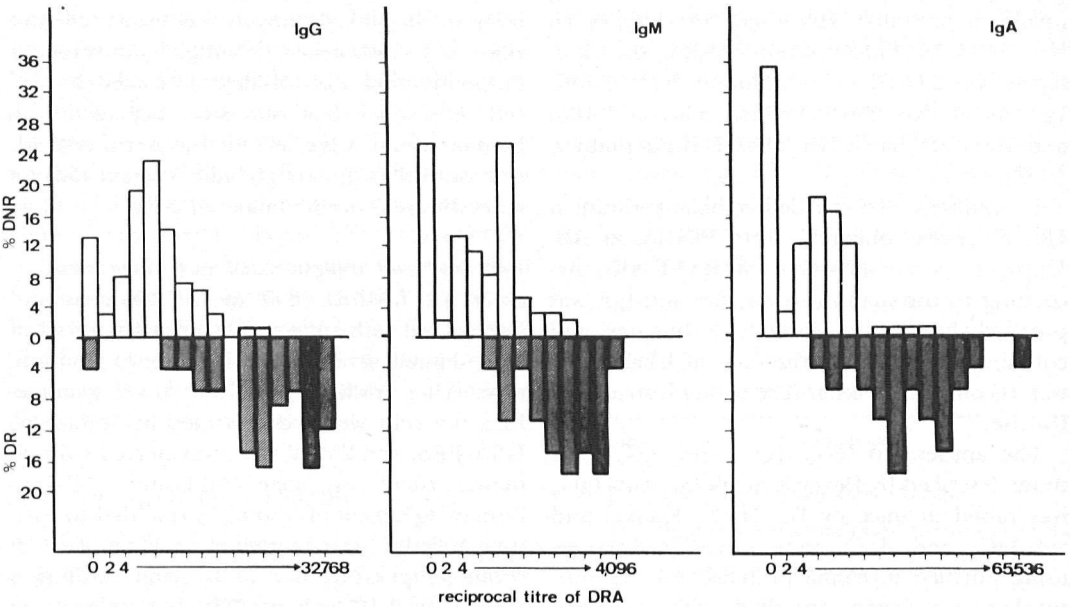


Fig. 1. Comparison of distribution of the serum titres of DRA in the IgG, IgM and IgA classes in dextran non reactors (DNR) and dextran reactors (DR). Open columns = DNR, hatched columns = DR. Samples were collected prior to the infusions.

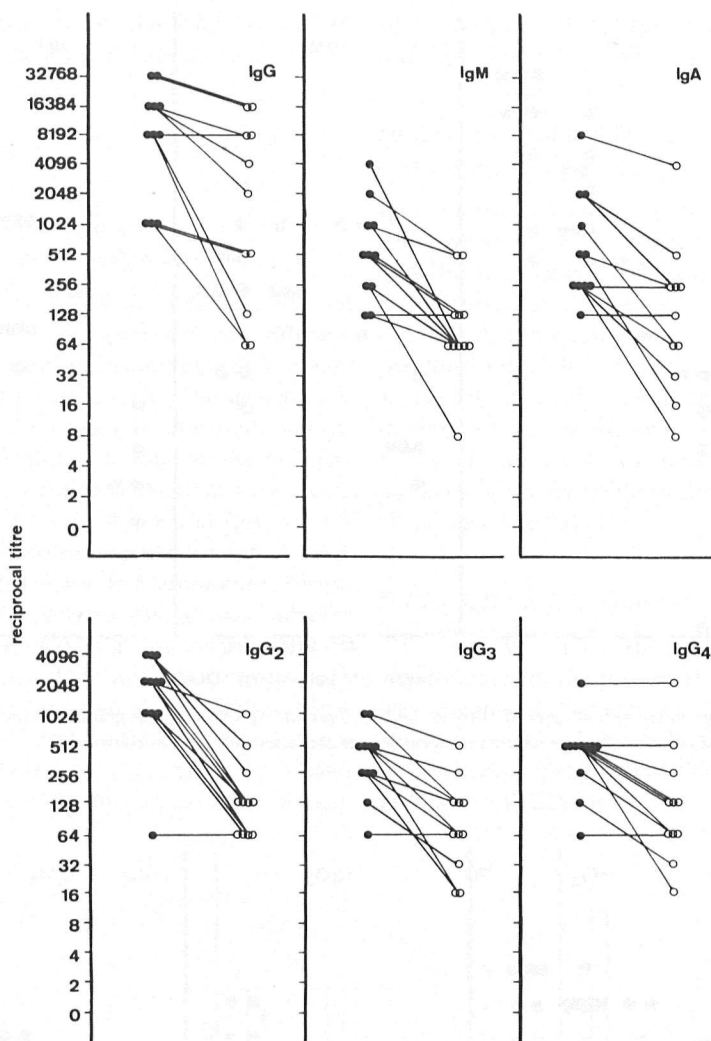


Fig. 4. Titres of DRA in the Ig classes and IgG subclasses before (●) and after (○) dextran infusion in patients with grade II to IV DIAR.

Investigating the sera from the DNR, two cases showed evidence for small amounts of dextran reactive IgE antibodies.

DRA of IgG subclasses in reactors and non reactors to dextran

Patients with DIAR of grades II to IV had the highest levels of IgG₂ antibodies. The patterns for IgG₃ and IgG₄ were similar except for slightly lower titres (Fig. 3). In grade I reactions titres were low for all IgG subclasses tested.

Significant discrepancies between different IgG subclasses were seen in 67% of individual sera tested, thus supporting the specificity of reaction. In DNR, antibody activity was found in all the IgG subclasses but the titres were much lower than in DR.

Influence of dextran infusion on DRA titres

Comparison of serum samples collected from patients with grade II to IV reactions before and after dextran infusion showed a variable

but significant decrease in DRA titres of IgG, IgM, and IgA classes and the IgG subclasses tested ($P < 0.01$ as checked by the sign-test). The magnitude of this effect was similar for DRA of IgG, IgM and IgA classes.

With respect to the IgG subclasses a more pronounced decrease was found for IgG₂ antibodies compared to IgG₃ and IgG₄ antibodies (Fig. 4, $P < 0.05$ as tested by the Friedman test). The degree of neutralization did not correlate to the volume of clinical dextran infused.

Titres of DRA in NHS pool

In the serum pool, the titres of DRA of IgG, IgM, and IgA classes were 64, 32, and 16 respectively. In 12 tests performed the titres never deviated more than ± 1 titre step.

DISCUSSION

A positive correlation has been shown to exist between titres of DRA estimated by passive haemagglutination and increasing severity of DIAR (10). However, the relative contribution of the different Ig-classes and IgG-subclasses to the antibody activity measured has not been known. The fact that most patients with severe reactions had precipitating DRA in their sera prior to DIAR suggested that DRA of IgG-class were of predominant importance (9, 15). This is confirmed by results of the present study with a modified RCLAAR showing a strong positive correlation between titre of IgG and severity of DIAR with the four lethal cases confined to the highest titre range. A less pronounced positive correlation and generally lower titres were found for IgA and IgM. It is concluded that patients with severe DIAR are high responders to dextran, expressing antidextran activity in the IgG, IgA, and IgM classes.

The technique of the modified RCLAAR (17) is very dependent on the specificity of the antiglobulins used and also on the method by which the red cells are coated with antigen. In this work the specificity of the antiglobulin sera was carefully controlled by coating cells with purified myeloma proteins and optimal dilutions were selected at which no cross-reactions

occurred. Dextran molecules were attached to the erythrocyte membrane by lipophilic stearyl groups which penetrate the lipid layer of the membrane without damage, therefore preventing non-specific globulin uptake.

The concentration of DRA of IgG-class is high in patients with grade III and IV reactions (c. 400 $\mu\text{g/ml}$, unpublished data). These patients received 1–10 ml of clinical dextran, before discontinuation of the infusion. One ml of clinical dextran already represents a large dose of antigen (60–100 mg) capable of generating several hundred mg of immune complexes in the patient's circulation. The fact that the degree of neutralization of DRA does not correlate to the volume of dextran infused may reflect varying affinity of DRA. The decrease of DRA titres of 1–7 steps (Fig. 4) produced by dextran infusion in DR implies that 50–99% of free antibodies have been converted to immune complexes. The circumstances favour formation of large complexes, formed at or near equivalence, as evidenced e.g. by a significant decrease of Clq levels in patients with severe DIAR (8). Further, patients with fatal DIAR show widely disseminated occlusive material in the vasculature of the lung (31, T. Saldeen, personal communication). This material consists of hyaline globuli with staining properties of fibrin and accumulations of leukocytes and thrombocytes. Findings accord with deposition of immune complexes and activation of the coagulation sequence and correspond to the picture seen in experimental immune complex-, but not cytotoxic anaphylaxis in monkeys (29). In conclusion, we consider available data as evidence for type III mechanisms going on during DIAR. Other examples of type III anaphylaxis are represented by patients with deficiency of IgA (18) or factor IX (23).

No evidence of circulating DRA of the IgD or IgE class in DR was obtained by RCLAAR and RCLA*AR. The IgE results accord with earlier data obtained by radioallergosorbent technique and passive cutaneous anaphylaxis in monkeys, as well as negative skin tests in 68% of DR (10). These results are supported by the fact that dextran is a thymus-independent antigen and that IgE-responses are thymus-dependent (H.

Wigzell, personal communication). Penicillin allergy and insect venom hypersensitivity are clinical states in which thymus-dependent antigens can induce IgE-class antibodies (1). The significance of the weakly positive IgE results found in our study in sera of two patients who tolerated dextran infusion, is uncertain. Furthermore, no increased plasma histamine levels could be demonstrated in patients with severe DIAR (20) and no histamine release from leukocytes of DR has been found (16). In conclusion, no evidence is available for DRA of IgE-class or mast cell-derived mediators of anaphylaxis due to type I reactions in DIAR.

DRA of the IgG subclasses IgG₂, IgG₃, and IgG₄ were found both in DR and DNR. Yount et al. (30) have shown that immunization of man with native dextran or other polysaccharides led to an antibody response that was predominantly IgG₂. Our data accord with theirs since we found the highest titres and the most pronounced neutralization in the IgG₂ subclass in DR, particularly in patients with severe DIAR. The latter finding indicates that IgG₂ plays an important role. With regard to IgG₄, rather high DRA titres (64–2,048) were found in DR. On the other hand, no evidence for short-term antibodies was demonstrable in sera of DR by passive cutaneous anaphylaxis in monkeys (10). Because of the relatively low sensitivity of the latter technique, it can not be excluded that IgG₄-DRA are present on mast cells in DR and contribute to elicitation of DIAR. IgG₁ could not be measured because of the cross-reactivity of the antisera. Indirect measurement of specific IgG₁ by calculating the total IgG antibodies minus the values for specific IgG₂, IgG₃ and IgG₄, gives evidence that this subclass of DRA may also be contributory as elicitor. However, this problem should be investigated by the use of monoclonal antibodies against IgG₁.

Whereas DRA of IgG, IgA, and IgM classes occur at low titres in the great majority of DNR, a few of them have high titres (≥ 512). This means that individual predisposition to develop DIAR cannot be predicted from determination of DRA. However, the method of RCLAAR allows to delineate a risk group of

about 2% of potential reactors. Since the incidence of severe DIAR is estimated to be about 0.05% (Ljungström & Renck, personal communication) only 1 in 40 individuals with high titres develops DIAR. Theoretically, severe DIAR could be eliminated by screening of patients with RCLAAR or other methods (24) estimating DRA preferably of IgG class. Because of practical difficulties the alternative preventive measure of injection of monovalent haptendextran (Promit®, Pharmacia AB) prior to dextran infusion is to be preferred at present (21, 25).

ACKNOWLEDGEMENTS

The excellent technical assistance of Mrs. Renate Steiner is gratefully acknowledged. We thank Dr. L. Havelec (Institut für Medizinische Statistik und Dokumentation, University of Vienna) who kindly helped with the statistical analysis of the results.

This study was supported by grants from the "Dr. Adolf Schärf Fonds" and the "Theodor Körner Stiftungsfonds", Vienna, Austria.

REFERENCES

1. Becker, E. L. & Austen, K. F.: Anaphylaxis. In Miescher, P. A. & Müller-Eberhard, H. J. (eds.): Textbook of immunopathology, pp. 117–135, 2nd ed. Grune and Stratton, New York, 1976.
2. Coombs, R. R. A., Howard, A. W. & Mynors, L. S.: A serological procedure theoretically capable of detecting incomplete or non-precipitating antibodies to soluble protein antigens. *Br. J. Exp. Pathol.* 34, 525–534, 1953.
3. Devey, M. E. & Voak, D.: A critical study of the IgG subclasses of Rh anti-D antibodies formed in pregnancy and in immunized volunteers. *Immunology* 27, 1073–1097, 1974.
4. Gibbons, R. & Banghart, S.: Synthesis of extracellular dextran by cariogenic bacteria and its presence in human dental plaque. *Arch. Oral. Biol.* 12, 11–24, 1967.
5. Gold, E. R. & Fudenberg, H. H.: Chromic chloride: a coupling reagent for passive haemagglutination reactions. *J. Immunol.* 99, 859–863, 1967.
6. Grönwall, A.: Antigenicity of Swedish clinical dextran (Macrodex). *Acta Soc. Med. Upsal.* 64, 244–246, 1959.
7. Hämmerling, U. & Westphal, O.: Synthesis and use of O-stearoyl polysaccharides in passive haemagglutination and hemolysis. *Eur. J. Biochem.* 1, 46–50, 1967.
8. Hedin, H.: Dextran-induced anaphylactoid reactions in man. Immunological in vitro and in vivo studies. Diss., Acta Univ. Upsal. No. 432, 1–43, 1977.
9. Hedin, H. & Richter, W.: Pathomechanism of dextran-induced anaphylactoid/anaphylactic reactions in man. *Int. Arch. Allergy Appl. Immunol.* 68, 122–126, 1982.

10. Hedin, H., Richter, W. & Ring, J.: Dextran-induced anaphylactoid reactions in man. Role of dextran reactive antibodies. *Int. Arch. Allergy Appl. Immunol.* 52, 145–159, 1976.
11. Hedin, H., Richter, W., Messmer, K., Renck, H., Ljungström, K.-G. & Laubenthal, H.: Incidence, pathomechanism and prevention of dextran-induced anaphylactoid/anaphylactic reactions in man. *Dev. Biol. Stand.* 48, 179–189, 1981.
12. Jacobsson, L. & Zsiga, J.: Dextran-reacting antibodies in human serum. *Acta Soc. Med. Upsal.* 63, 165–179, 1958.
13. Kabat, E. A. & Bezer, A. E.: The effect of variation in molecular weight on the antigenicity of dextran in man. *Arch. Biochem. Biophys.* 78, 306–318, 1958.
14. Kabat, E. A. & Mayer, M.: *Experimental immunochemistry*, pp. 405–444, 2nd ed. Thomas, Springfield, 1961.
15. Kabat, E. A., Turino, G. M., Tarrow, A. B. & Maurer, P. H.: Studies on the immunochemical basis of allergic reactions to dextran in man. *J. Clin. Invest.* 36, 1160–1170, 1957.
16. Kohen, M., Mattikow, M., Middleton, E. & Butch, D. W.: A study of three untoward reactions to dextran. *J. Allergy* 46, 309–314, 1970.
17. Kraft, D., Wilson, D. V. & Devey, M. E.: Penicillin allergy studies by a modified red cell-linked antigen-antiglobulin reaction. *Int. Arch. Allergy Appl. Immunol.* 52, 248–256, 1976.
18. Leikola, J., Koistinen, J., Lehtinen, M. & Wirolainen, M.: IgA-induced anaphylactoid transfusion reactions: A report of four cases. *Blood* 42, 111–119, 1973.
19. Lewis, D. (ed.): *Dextran – 30 years*. Acta Univ. Upsal. Symp. 3, 1–132, 1977.
20. Lorenz, W., Doenicke, A., Messmer, K., Reimann, H. J., Thermann, M., Lahn, W., Berr, J., Schmal, A., Dormann, P., Regenfuss, P. & Hamelmann, H.: Histamine release in human subjects by modified gelatin (Haemaccel) and dextran; an explanation for anaphylactic reactions observed under clinical conditions? *Br. J. Anaesth.* 48, 151–159, 1976.
21. Messmer, K., Ljungström, K.-G., Gruber, U., Richter, W. & Hedin, H.: Prevention of dextran-induced anaphylactoid reactions by hapten inhibition. *Lancet* i, 975, 1980.
22. Neill, J. M., Hehre, E. J., Sugg, J. Y. & Jaffe, E. M.: Serological studies on sugar. I. Reactions between solutions of reagent sucrose and type II antipneumococcus serum. *J. Exp. Med.* 70, 427–442, 1939.
23. Nilsson, I. M., Jonsson, S., Sundqvist, S. B., Ahlberg, Å. & Bergentz, S. E.: A procedure for removing high titer antibodies by extracorporeal protein-A-Sepharose adsorption in hemophilia; substitution therapy and surgery in a patient with hemophilia B and antibodies. *Blood* 58, 38–44, 1981.
24. Palosuo, T. & Milgrom, F.: Appearance of dextrans and antidextran antibodies in human sera. *Int. Arch. Allergy Appl. Immun.* 65, 153–161, 1981.
25. Richter, A. W. & Hedin, H. I.: Dextran hypersensitivity. *Immunology Today* 3, 132–138, 1982.
26. Richter, W. & Kågedal, L.: Preparation of dextran-protein conjugates and studies of their immunogenicity. *Int. Arch. Allergy Appl. Immunol.* 42, 887–904, 1972.
27. Richter, W., Hedin, H., Ring, H., Kraft, D. & Messmer, K.: Anaphylaktoide Reaktionen nach Dextran. I. Immunologische Grundlagen und klinische Befunde. *Allergologie* 3, 51–58, 1980.
28. Ring, J. & Messmer, K.: Incidence and severity of anaphylactoid reactions to colloid volume substitutes. *Lancet* i, 466–469, 1977.
29. Smedegård, G.: Anaphylactic shock. Pathophysiology of aggregate and cytotoxic anaphylaxis in the monkey. Diss., Acta Univ. Upsal. No. 548, 1980.
30. Yount, W. J., Dorner, M. M., Kunkel, H. G. & Kabat, E. A.: Studies on human antibodies. IV. Selective variations in subgroup compositions and genetic markers. *J. Exp. Med.* 127, 633–646, 1968.
31. Ziegler, H. K.: Sektionsbefund bei Dextranzwischenfall. *Med. Klin.* 73, 1089–1090, 1978.

Address:

Dr. D. Kraft
Institute of General and Experimental Pathology
Währingerstrasse 13
A-1090 Vienna
Austria

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.