All the groups of mice produced antibody towards the homologous coxsackievirus used for infection. There was no correlation between the extent of this response and depression of immune response towards heterologous antigens.

Discussion. Coxsackieviruses display a prominent age-dependent pathogenicity in mice, adults being relatively resistant. Nevertheless, infection of adult mice with 6 out of the 9 serotypes examined exerted a significant depression of the immune reactivity of the host to unrelated antigens. Coxsackieviruses A-15, B-1, B-2, B-4 and B-6 depressed the antibody response to either SRBC or poliovirus 1 or both and coxsackievirus B-3 depressed the cell-mediated contact sensitivity to oxazolone, as well as the humoral responses.

The data collected so far do not give much insight on the mechanism of the immunodepression observed. The lack of correlation between occurrence of immunodepression and extent of antibody response to the coxsackievirus used for infection seems to exclude immunological competition and suggests a less aspecific mode of action. Coxsackieviruses have been detected in the spleen[14–17] and in lymph nodes[14] of adult mice for several days after infection, but it is not known whether the presence of virus results from local replication or from drainage of other organs. In one study[17], no microscopical lesions suggestive of virus replication were seen in the spleen; in another study[18], extensive degeneration of the lymphoid elements of the thymus were observed but were attributed to intercurrent reovirus infection. Further studies that might clarify the mechanism of immunodepression are underway in this laboratory.

Whatever the mechanism, young mice, which are more sensitive to coxsackieviruses, should conceivably be more severely immunodepressed than adult mice. In this context it should also be considered that the process of immunological maturation with ageing can be more drastically impaired by viral infections than fully established immunological reactivity[12].

An impairment of immune functions might occur also in the course of human coxsackievirus infections and so explain the clinical observations referred to in the introduction. Moreover, it might help to explain the poor serological responses to live poliovirus vaccines in children excreting enteroviruses[19], a phenomenon that so far has not been satisfactorily understood[20]. Coxsackieviruses have been implicated in the aetiology of congenital heart malformations[21]. The present findings suggest that infection of the embryo with these viruses might also be responsible for congenital immunological defects, in analogy to what can happen in intrauterine rubella infection[22].

Summary. Adult mice infected with coxsackieviruses A-15, B-1, B-2, B-4 and B-6 showed depressed antibody responses to unrelated antigens; mice infected with coxsackievirus B-3 developed reduced humoral and cell-mediated immune responses. These findings might have clinical and epidemiological implications.

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Antigenic Heterogeneity among Monoclonal IgM: Observations on Guinea-Pig Sera

Antigenically distinct subclasses of immunoglobulins have been delineated for IgG[1,2] and IgA[3–5], and found to depend on structural differences. The situation regarding IgM is, however, still unclear. Antigenic differences have been reported by several investigators[6–10], but no correlation between the groups found in different laboratories has emerged. In the present study, antigenic subgroups were sought, utilizing monoclonal components from human sera and antisera raised in guinea-pigs, since observations with α_2-macroglobulin antisera had indicated guinea-pigs to be more sensitive to antigenic differences in primates, than are rabbits[11].

Material and methods. Sera from 54 patients with electrophoretically detected IgM bands were used, which had been kept frozen at −20 °C for periods up to 5 years. M-components were isolated by preparative electrophoresis in agarose gel, followed by gel filtration on Sephadex G 200. Immunoelectrophoresis and Ouchterlony double diffusion was performed in 1% agarose on 25 × 75 mm glass slides. Guinea-pigs in groups of 3 were immunized by s.c. injection with 50 µg of antigen in complete Freund's adjuvant at weekly intervals. In all, 9 antigens were used for immunization. The animals were sacrificed 4 weeks after the start, and exsanguinated by heart puncture. The yield was up to 12 ml of blood. Absorptions were performed at 37 °C for 1 h and 4 °C for 15 h. 2 animals in each of 2 groups were lost during the immunization and thus left no antiserum.

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Results. All antisera gave strong precipitation lines with purified normal IgM and also reacted with all tested macroglobulinemic sera. In addition they showed individual specificity for the IgM used as antigen, as demonstrated by spur formation in the Ouchterlony diffusions. By absorption with increasing amounts of normal human serum, one could get antisera reacting only with the M-component used for immunization. By absorbing with serum AP from a patient with agammaglobulinemia, one could obtain antisera reacting only with IgM, but still reacting with all sera in the panel of M-components.

When comparing 2 antisera after absorption with AP (Figure 1), it was noted that anti-MO-8 only showed a faint reaction with EE, whereas anti-EE still strongly reacted to all other sera. Anti-MO-8 was then further absorbed with serum EE, and this resulted in an antiserum that no longer gave a precipitation line with normal serum. This antiserum was then reacted with all 54 sera containing monoclonal IgM-components. With 5 of these a strong precipitation reaction remained after the absorption (Figure 2). Furthermore 4 sera reacted weakly, with short fuzzy precipitation lines (Figure 2). No precipitates were obtained with 44 sera. No correlation was found between reactivity and concentration of the M-component, light chain type, electrophoretic mobility, or cryoprecipitability. None of the reacting sera had cold agglutinin activity. The strong reactors all had typical symptoms of Waldenström's macroglobulinemia.

Among the weak reactors, 2 had an unusual syndrome with high titre rheumatoid factor, intermediate complexes and polyclonal IgG increment in addition to IgM-components. It may be of interest that these M-components seem to share idiotypic determinants[12]. One further case had a positive Waaler-Rose test.

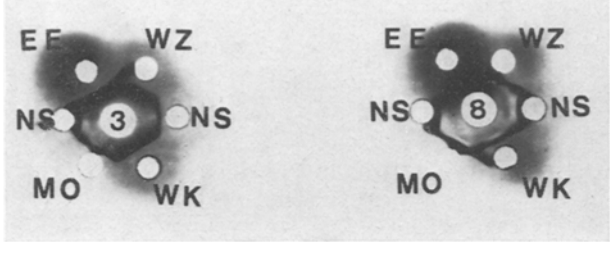

Fig. 1. Ouchterlony pattern of antiserum 3, anti-EE, and antiserum 8, Anti MO, after absorption with γ-globulin-serum AP.

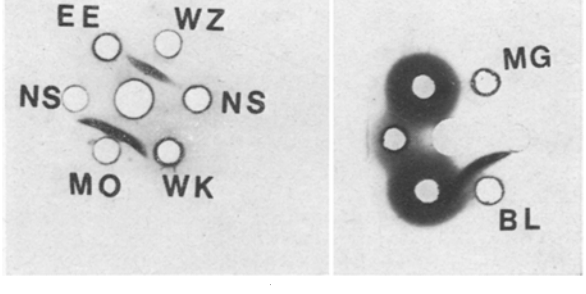

Fig. 2. Ochterlony diffusion of antiserum anti-MO absorbed with AP and EE. Strong reactions are seen with MO, BL and WZ. A faint reaction remains with MG. Normal serum (NS) and 6 other macroglobulin sera do not react with the antiserum.

It was possible to absorb the 2 other guinea-pigs immunized with 'MO' in the same way as anti-MO-8. However, reverse absorption of anti-EE with MO completely abolished reactivity with IgM. We also failed to absorb 3 anti-MO-sera raised in rabbits to show the reactivity of anti-MO-8.

Discussion. The present study supports again the exsitence of antigenic subclasses in human IgM, and seems to indicate that guinea-pigs are more suitable for detecting minor antigenic differences among human plasma proteins than rabbits. This hypothesis, however, needs further support. The disadvantage caused by the limited amount of serum obtained from each animal, can apparently be overcome by using a number of animals in each immunization, since they seem to react in a more uniform and predictable fashion than rabbits.

The difficulties in delineating IgM subgroups with precipitating antisera have led to the search for other systems of classification. Thus variations in the interaction with Cl_q has lead to the distinction of 2 groups[13] related to the degree of coiling in the Fc region[14]. WELLS et al.[15] used a hemagglutination inhibition reaction where a panel of $CrCl_3$-treated red cells were coated with monoclonal IgM. Antibodies to IgM present in one patient serum agglutinated such cells in high titers, and this reaction could be inhibited by a proportion of both M-components and normal sera, thus representing an allotypic marker. A similar system has been used previously[16]. Finally HARBOE et al.[17] could define 2 groups for both IgM and IgA components, based on different binding to protein A from staphylococci. Attempts have so far failed to correlate all these recognition systems with each other. It is felt that they are the consequence of structural variations in the large IgM molecule. Their biological significance remains to be elucidated.

Summary. Antisera against purified monoclonal IgM from patients with Waldenström's macroglobulinemia were raised in guinea-pigs. Absorption with normal human serum and heterologous macroglobulinemic serum resulted in an antiserum to a subgroup present in 5 of 54 sera.

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