PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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A. A. Holder and R. R. Freeman

Phil. Trans. R. Soc. Lond. B 1984 **307**, 171-177
doi: 10.1098/rstb.1984.0117

Phil. Trans. R. Soc. Lond. B **307**, 171–177 (1984) [171]
Printed in Great Britain

Protective antigens of rodent and human bloodstage malaria

By A. A. Holder and R. R. Freeman

Department of Molecular Biology, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, U.K.

Bloodstage malaria parasites are antigenically complex, but individual antigens can be identified and analysed using monoclonal antibodies. Two monoclonal antibodies that recognize a 235000 molecular mass *Plasmodium yoelii* rhoptry protein provide some protection when injected into mice against a challenge infection. The purified rhoptry protein also provides protective immunity against *P. yoelii* YM when used to vaccinate mice and fulminating infections are converted into self-limiting, reticulocyte-restricted infections. Another monoclonal antibody immunoprecipitates a 230000 molecular mass protein and a series of proteolytic processing fragments. At least one of these processing fragments, probably a 90000 molecular mass species, is located on the merozoite surface. Mice immunized with the purified protein were protected against challenge infection with *P. yoelii* YM. This antigen may provide protection by inducing a cell-mediated immune response. A monoclonal antibody raised against *P. falciparum* schizonts reacts with a 195000 molecular mass protein which is synthesized in schizonts and subsequently cleaved. Fragments of the 195000 molecular mass protein are expressed as major antigens on the merozoite surface. The 195000 molecular mass *P. falciparum* protein and the 230000 molecular mass *P. yoelii* protein belong to a class of malaria parasite antigens which probably is important in the induction of a protective immune response in the host.

Introduction

In hyperendemic areas, those individuals who survive their initial *Plasmodium falciparum* infections during childhood acquire an effective immunity against this malaria parasite which serves them throughout their adult lives. The physiological factors contributing to the development of a state of immunity to malaria are not fully understood, but it seems, from immunoglobulin transfer studies (Cohen *et al.* 1961), that antibodies directed against antigens of the bloodstage forms of the parasite play an important part. A crude vaccine, comprising blood schizonts and merozoites emulsified in an adjuvant, has been shown to induce protective immunity against *P. falciparum* infection in owl monkeys (Siddiqui *et al.* 1981). For further development of a vaccine against falciparum malaria it is necessary to identify those antigenic components of the parasite which induce protective immune responses in the host. Bloodstage malaria parasites are antigenically complex, but individual antigens can be identified and analysed using monoclonal antibodies. There are several distinct hypotheses regarding the likely nature of the bloodstage protective antigens (Cohen *et al.* 1969; Miller *et al.* 1975; Hommel *et al.* 1983; Udeinya *et al.* 1983), and so it is possible to design a range of different strategies for producing monoclonal antibodies which may result in the identification of important parasite antigens. It is also possible to use animal models, for instance *P. yoelii* or *P. chabaudi* infections in mice, to identify classes of protective antigens that may be common to malaria parasites in general, and then to apply the knowledge gained to *P. falciparum*. It has been established that some of the protective antigens of malaria parasites are associated with the merozoite forms, that is, the extracellular forms that invade erythrocytes. This has been shown most clearly by the successful immunization of rhesus monkeys with purified *P. knowlesi* merozoites (Mitchell *et al.* 1975), and by observation of the interaction of antibodies from immune humans with *P. falciparum* merozoites *in vitro* (Green *et al.* 1981). We have chosen, therefore, to study the antigens of malaria merozoites, and we shall describe the characterization of some of the merozoite antigens of *P. yoelii* and *P. falciparum*. The antigens of the rodent parasite are known to be involved in the induction of protective immunity, while the protective nature of the *P. falciparum* antigen concerned remains to be demonstrated.

Protective antigens of rodent malaria parasites

A rhoptry protein of P. yoelii

Two monoclonal antibodies recognizing *P. yoelii* merozoites were found to convert fulminating infections into self-limiting infections when injected into infected mice (Freeman *et al.* 1980). Both antibodies immunoprecipitated the same antigen, a 235000 molecular mass protein. The protein was purified by monoclonal antibody–Sepharose affinity chromatography, and was shown to provide some protective immunity against *P. yoelii* when used to vaccinate mice. Again, challenge infections that were lethal in controls were converted into self-limiting infections in the immunized mice (Holder & Freeman 1981). The 235000 molecular mass protein has been localized to the rhoptries of *P. yoelii* merozoites by immunoelectron microscopy (Oka *et al.* 1984).

The rhoptries are paired organelles located at the apical end of the merozoite. It has been suggested that they secrete a substance at the point of merozoite–erythrocyte attachment which assists penetration, perhaps by inducing invagination of the red cell membrane (Ladda 1969; Aikawa *et al.* 1978). The observation that antibodies specific for the 235000 molecular mass rhoptry protein can interfere with the invasion of mouse erythrocytes by *P. yoelii* merozoites *in vivo* is consistent with this suggestion, but the present lack of an *in vitro* cultivation technique for *P. yoelii* has precluded further analysis of the function of this protein.

In vivo, the conversion of fulminating to self-limiting infections in mice immunized with the 235000 molecular mass protein and challenged with the YM strain of *P. yoelii* is accompanied by, and possibly caused by, a change in the parasite's host cell preference. In non-immunized mice, *P. yoelii* YM invades both reticulocytes and mature erythrocytes, but in immunized mice parasitaemia is apparently restricted to reticulocytes. Knowles & Walliker (1980) have isolated avirulent subclones of *P. yoelii* YM which are reticulocyte-restricted, from a cloned virulent line of this parasite, and it is possible that antibodies against the 235000 molecular mass protein select such variants. Alternatively, the presence of antibodies against the rhoptry protein may reduce the invasiveness of all merozoites below the level required for successful invasion of mature erythrocytes, but not effectively enough to prevent invasion of reticulocytes. This suggestion assumes that invasion of reticulocytes by *P. yoelii* merozoites is a more efficient process than invasion of mature erythrocytes, perhaps because of the properties of the reticulocyte membrane, or because invasion of reticulocytes can occur in the venous sinuses of the bone marrow, where schizonts are in intimate contact with uninfected reticulocytes (Weiss 1983).

An homologous protein has been detected in the 17X strain of *P. yoelii*. This strain produces self-limiting, reticulocyte-restricted infections in mice. Nevertheless, it also synthesizes the 235000 molecular mass rhoptry protein, and the monoclonal antibodies specific for this protein do not affect the course of *P. yoelii* 17X infections. The epitopes recognized by the two protective monoclonal antibodies are restricted among the rodent malaria parasites and are not present in *P. falciparum* (table 1).

A merozoite surface protein of P. yoelii

One of our anti-*P. yoelii* monoclonal antibodies, 25.1, reacted with schizonts and merozoites in the immunofluorescence test, but did not provide passive protection when injected into mice infected with *P. yoelii* (Freeman *et al.* 1980). This antibody immunoprecipitated a 230000 molecular mass protein, and a series of proteolytic processing fragments derived from it, from extracts of parasitized mouse erythrocytes, and could be used to purify these related polypeptides on a large scale by affinity chromatography (Holder & Freeman 1981). Mice immunized with the purified protein were protected against challenge infection with *P. yoelii* YM: parasitaemias in immunized mice were brief and low grade, and clearance was associated with the appearance of 'crisis forms' in blood smears. Immunization before challenge did not affect the host cell preference of the parasite.

TABLE 1. CROSS-REACTION WITH OTHER MALARIA SPECIES OF THE MONOCLONAL ANTIBODIES SPECIFIC FOR THE 235000 MOLECULAR MASS *PLASMODIUM YOELII* RHOPTRY PROTEIN

Plasmodium species	indirect immunofluorescence reactivity antibody 25.37	 antibody 25.77
P. yoelii yoelii YM	+	+
P. yoelii yoelii 17X	+	+
P. yoelii yoelii 33X	+	+
P. yoelii killicki	−	+
P. yoelii nigeriensis	+	+
P. vinckei vinckei	−	−
P. v. brucechwatti	−	−
P. vinckei lentum	−	−
P. vinckei petteri	−	+
P. chabaudi chabaudi	−	+
P. chabaudi adami	−	+
P. falciparum	−	−

Serum from mice immunized with the 230000 molecular mass antigen had a high antibody titre against the antigen, but was not protective on passive transfer (Freeman & Holder 1983*a*). On the other hand, serum from mice recovered from *P. yoelii* infection, while showing an equivalent antibody titre against the 230000 molecular mass antigen protected mice completely in passive transfer experiments. It was concluded that this antigen may provide protection against *P. yoelii* by induction of a cell-mediated immune response as described by Playfair *et al.* (1979), the end result of which is release of toxic factors from liver macrophages which cause intraerythrocytic death of the parasites (Clark & Hunt 1983; Dockrell & Playfair 1983). In one experiment we have investigated the specificity of protective immunity afforded to BALB/c mice by immunization with the 230000 molecular mass protein purified from *P. yoelii* YM. Protection against the homologous parasite, *P. yoelii* YM, was complete (figure 1). Partial protection was observed in mice challenged with *P. yoelii* 17X, *P. vinckei petteri* or *P. chabaudi*, in that peak parasitaemias were lower in immunized mice than in the controls. These results may reflect a variation between species and strains of rodent malaria parasites in susceptibility to intraerythrocytic killing, as has been suggested by Clark *et al.* (1977). They may also explain the antigenic basis of the partial cross-immunity observed in mice recovered from blood infections with these parasites (Cox 1970). A polyvalent antiserum raised in mice against the *P. yoelii* YM 230000 molecular mass antigen cross-reacted with all subspecies of *P. yoelii*, *P. vinckei* and *P. chabaudi* tested by immunofluorescence (Holder & Freeman 1984*a*).

If the 230000 molecular mass antigen induces protective immunity via the cell-mediated pathway as suggested, it need not be located on the merozoite surface: it could be as effective in turning on this type of response if it was secreted or shed into the plasma. An immunoelectron microscopy study has indicated that at least one of the processing fragments derived from the 230000 molecular mass protein is located on the merozoite surface (Oka *et al.* 1984), and surface-labelling analysis suggests that it is a fragment of 90000 molecular mass (Holder & Freeman 1984*a*). It is possible that this fragment is shed from the merozoite surface during invasion of the red cell, as *P. yoelii* ring forms do not react with antibody 25.1 by immunofluorescence.

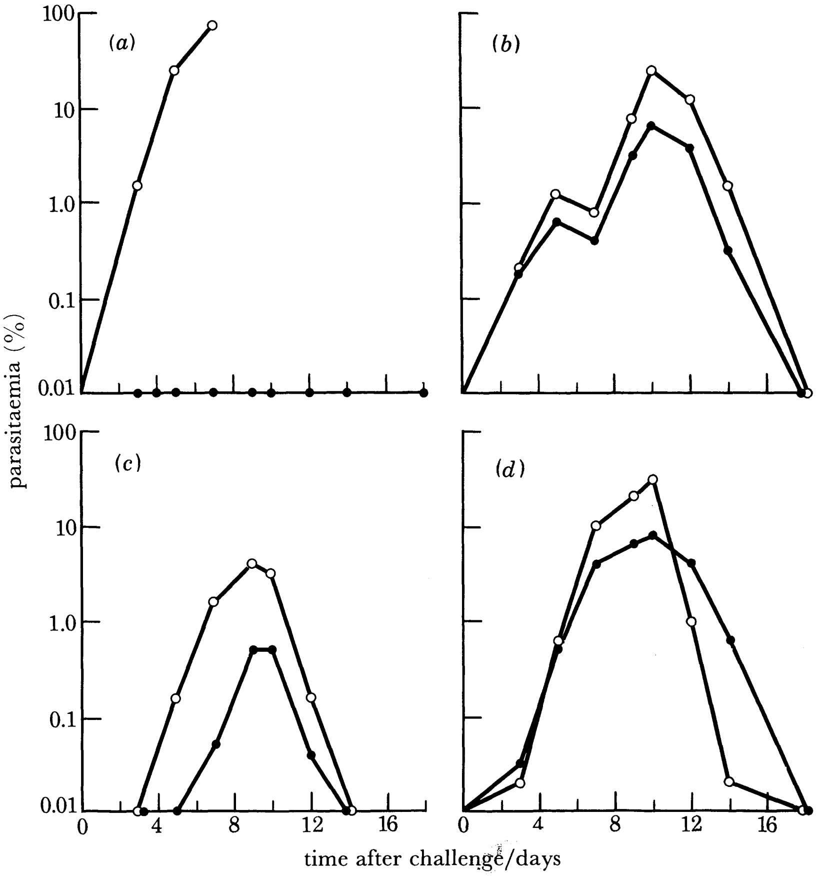

FIGURE 1. Specificity of the protective immunity afforded by immunization with the 230000 molecular mass protein purified from *P. yoelii* YM. Groups of four BALB/c mice were immunized intraperitoneally with 50 μg of the antigen in Freund's complete adjuvant and were boosted with 80 μg of the antigen in Freund's complete adjuvant 15 weeks later. Twenty-three days after the boost the mice were challenged with (*a*) *P. yoelii* YM, (*b*) *P. yoelii* 17X, (*c*) *P. vinckei petteri* 2CR, or (*d*) *P. chabaudi* 2AS, by intravenous inoculation of 10000 parasitised erythrocytes. Mean parasitaemias after immunization with the 230000 molecular mass *P. yoelii* YM protein and its fragments (●) or saline (○).

Serological cross-reaction between the *P. yoelii* 230000 molecular mass protein and a 195000 molecular mass protein of *P. falciparum* has been demonstrated (Holder *et al.* 1983).

BLOOD-STAGE ANTIGENS OF *P. FALCIPARUM*

For antigenic analysis leading to the development of a vaccine against human malaria, animal models no longer offer real advantages over the direct study of *P. falciparum* cultivated in human erythrocytes *in vitro*. The original culture method of Trager & Jensen (1976) has been modified and improved to enable large-scale production and higher yields. Parasite development can be synchronized easily using sorbitol (Lambros & Vanderberg 1979), and naturally released merozoites can be harvested from culture supernatants for antigenic analysis (Freeman & Holder 1983*b*; Heidrich *et al.* 1983). Serum from immune individuals can be used to identify subsets of *P. falciparum* proteins likely to contain the protective antigens of the parasite (Kilejian 1980; Perrin *et al.* 1981; Holder & Freeman 1982; Brown *et al.* 1982; Freeman & Holder 1983*b*; Heidrich *et al.* 1983), and monoclonal antibodies specific for these antigens can be produced (Perrin & Dayal 1982; Holder & Freeman 1982; Hall *et al.* 1983). Figure 2 illustrates how the combination of these techniques can assist in the analysis of *P. falciparum* antigens.

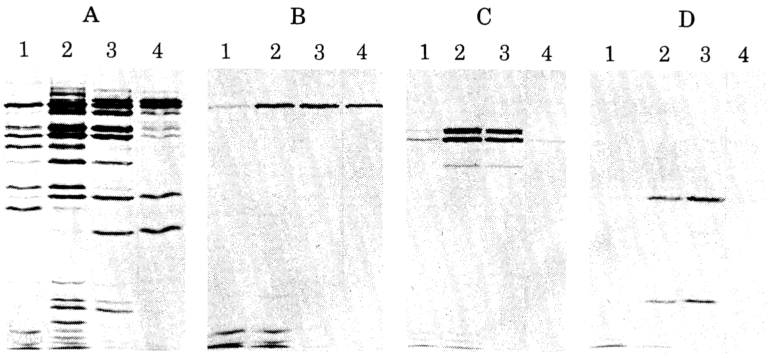

FIGURE 2. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis analysis of antigens immunoprecipitated from synchronous cultures of *P. falciparum*. Cultures were synchronized by two treatments with sorbitol and then pulse-labelled with [^{35}S]methionine for 30 min at 30 h (tracks 1), 36 h (tracks 2), 42 h (tracks 3) and 48 h (tracks 4) after the second sorbitol treatment. Detergent extracts were prepared and used for immunoprecipitation with (*a*) serum from immune humans, (*b*) monoclonal antibody 89.1, (*c*) monoclonal antibody 61.3, and (*d*) monoclonal antibody 209.3.

A merozoite surface protein of P. falciparum

A mouse monoclonal antibody, 89.1, raised against *P. falciparum* schizonts, reacted with schizonts and merozoites in the immunofluorescence assay, and immunoprecipitated a 195000 molecular mass polypeptide from detergent extracts of [^{35}S]methionine pulse-labelled schizonts (figure 2). Cleavage of this protein into several fragments late in schizogony was demonstrated by pulse-chase analysis and peptide mapping (Holder & Freeman 1982). By surface labelling of free merozoites followed by solubilization and immunoprecipitation, it was shown that an 83000 molecular mass fragment carrying the epitope recognized by antibody 89.1, is cleaved from the 195000 molecular mass precursor, and expressed as a major antigen on the merozoite surface (Freeman & Holder 1983*b*).

Recent results indicate that two additional cleavage fragments of 42000 and 19000 molecular mass are also located on the surface of *P. falciparum* merozoites, but they do not carry the epitope recognized by antibody 89.1 (Holder & Freeman 1984*b*). Taken together, these results suggest that the three major surface antigens of *P. falciparum* merozoites that are immunoprecipitated by human immune serum are derived by processing of a common, high molecular mass precursor protein. As surface antigens, these polypeptides could be the targets of protective antibodies capable of blocking invasion or agglutinating merozoites.

A CLASS OF MEROZOITE SURFACE ANTIGENS

The 195000 molecular mass protein of *P. falciparum* is clearly homologous to the 230000 molecular mass *P. yoelii* protein. Cross-reactive antigens have been detected in *P. vinckei* and *P. chabaudi*, and in *P. chabaudi* the antigenic protein has been identified as a 250000 molecular mass protein, synthesized in schizonts (Holder *et al.* 1983; Boyle *et al.* 1982). The function of this class of proteins is unknown, but their location (in processed form) over the entire merozoite surface suggests that they may be involved in red cell recognition. Perhaps the processing that occurs during merozoite formation results in activation of the protein or facilitates shedding during invasion of the red cell. Whatever their function, the evidence from the rodent models, and the characteristics of the proteins, indicate that this class of antigens can induce a protective immune response in the host.

CONCLUSIONS

During its asexual multiplication in red cells the malaria parasite synthesizes a complex range of antigens. The identification and analysis of those antigens that are important in the induction of protective immune responses, further our understanding of the interaction of the parasite with its mammalian host and are crucial steps in the development of a vaccine against the disease.

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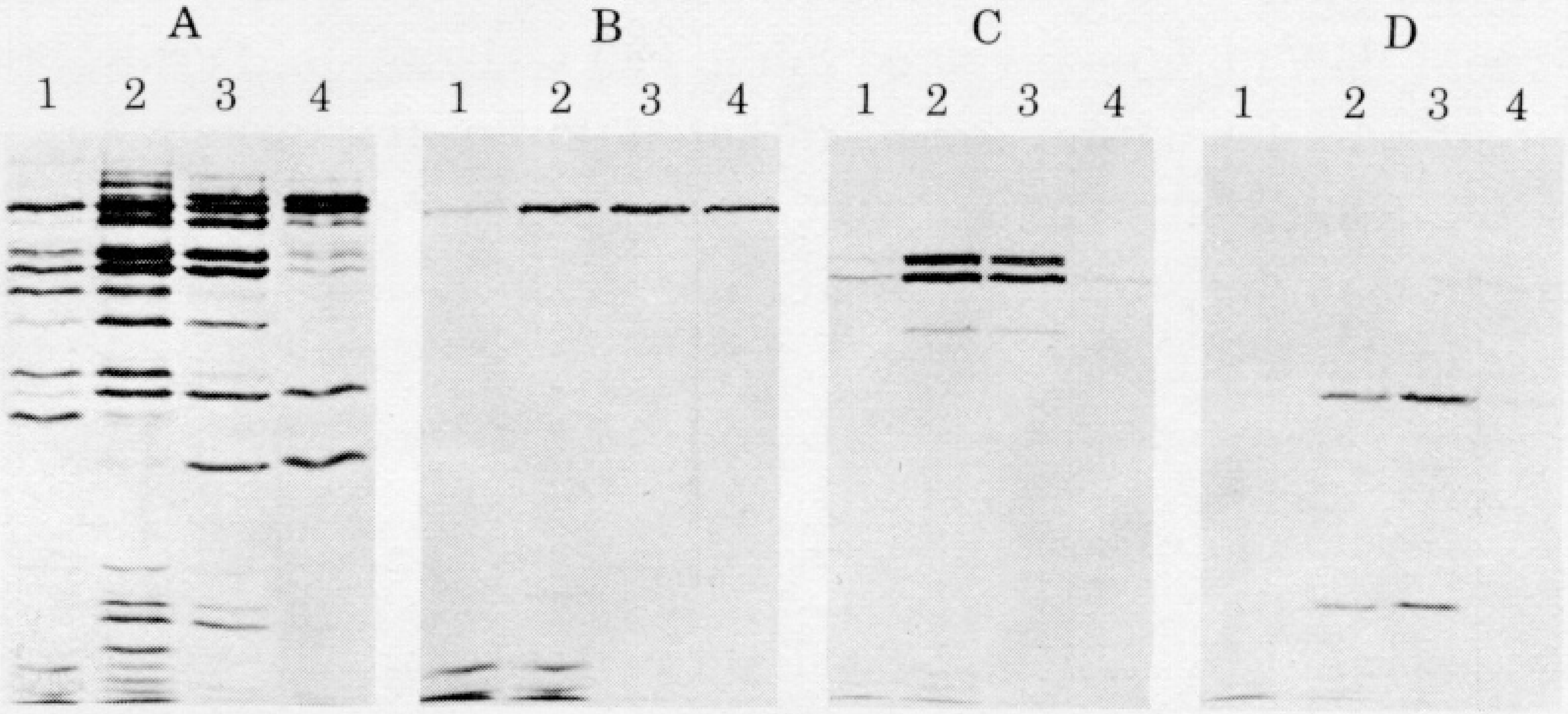

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