neutrophil plasma membrane. The neutrophil membrane has previously resisted attempts at purification because of contamination with the large number of intracellular membrane-bound organelles and the absence of any clear enzyme marker.

The marked immunogenicity and abundance of the carbohydrate on tumour cells which has resulted in this large expression of monoclonal antibodies with similar specificity, coupled with the notable stability of the antigen to most fixation techniques, has raised the possibility of the use of these antibodies in tumour diagnosis.

The antigen recognized by MC1-4 has been shown to be expressed in large amounts on several types of tumour, especially adenocarcinoma of the stomach, colon, breast and lung. Expression of the antigen on normal colon and breast is restricted to mucin or secretions whilst in the stomach expression is restricted to readily identifiable cell types. The antigen is not expressed at all in normal adult lung though it is detectable in the developing alveolar buds in foetal lung. A limited survey suggests that these monoclonal antibodies compare with other unrelated monoclonal and polyclonal antibody tumour markers. Increased antigen expression is usually associated with malignancy. However, expression may also be found in normal and benign tissues. It is likely that antigen expression in benign tissues reflects some aspect of cellular differentiation and is likely to be a transient phenomenon.

Stage-specific antigens of *Trichinella spiralis*

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Species which make up the phylum Nematoda include those which cause major chronic debilitating diseases. However, studying the pathogenesis of these parasites is hampered by their restricted host range and by their ability to develop into adult parasites in tissues remote from the site of infection. In recent years, work on the nematode parasite *Trichinella spiralis*, which causes trichinellosis, has suggested that it may have potential as a model for the study of other nematodes.

Recent work indicates that *T. spiralis* is not unique. Many parasites, both unicellular and multicellular, pass through a series of discrete developmental life cycle stages. Such stages are normally characterized by stage-specific surface antigens and frequently, but not always, accompany a change in the host environment. This has recently been shown for the parasitic nematode *T. spiralis* (Philipp et al., 1980; Clark et al., 1982). In this organism the surface can be labelled with radioactive iodine to yield very few (one to five) different electrophoretic components, which are different for each stage of the parasite and which are usually glycosylated. The stage-specific surface proteins of nematodes are also specific in converting the larval form of the parasite to a form capable of invasion of the host tissues.

Lessons learnt with this easily maintained laboratory model may thus have application in the study of other nematodes. *T. spiralis* is not unique. Many parasites, both unicellular and multicellular, pass through a series of discrete developmental life cycle stages. Such stages are normally characterized by stage-specific surface antigens and frequently, but not always, accompany a change in the host environment. This has recently been shown for the parasitic nematode *T. spiralis* (Philipp et al., 1980; Clark et al., 1982). In this organism the surface can be labelled with radioactive iodine to yield very few (one to five) different electrophoretic components, which are different for each stage of the parasite and which are usually glycosylated. The stage-specific surface proteins of nematodes are also specific in converting the larval form of the parasite to a form capable of invasion of the host tissues.

The antigen recognized by MC1-4 has been shown to be expressed in large amounts on several types of tumour, especially adenocarcinoma of the stomach, colon, breast and lung. Expression of the antigen on normal colon and breast is restricted to mucin or secretions whilst in the stomach expression is restricted to readily identifiable cell types. The antigen is not expressed at all in normal adult lung though it is detectable in the developing alveolar buds in foetal lung. A limited survey suggests that these monoclonal antibodies compare with other unrelated monoclonal and polyclonal antibody tumour markers. Increased antigen expression is usually associated with malignancy. However, expression may also be found in normal and benign tissues. It is likely that antigen expression in benign tissues reflects some aspect of cellular differentiation and is likely to be a transient phenomenon.

**Abbreviation used:** SDS, sodium dodecyl sulphate.
can, in addition, change within a stage (Philipp et al., 1980; Jungery et al., 1983; Ortega-Pierres et al., 1983) as well as between stages. Finally, and as further evidence for the dynamic nature of the nematode surface, there is evidence for release, and hence turnover, of the stage-specific surface antigens (Philipp et al., 1980; Ortega-Pierres et al., 1983).

Significantly, similar studies with other nematodes also reveal few antigenic components by surface-labeling techniques, although absolute stage specificity as in the example of *T. spiralis* is not always observed (Maizels et al., 1983a,b,c; Philipp et al., 1984).

Analysis of the structure of parasites at a molecular level has two aims: the identification of potentially protective antigens, and the identification of molecules which may be used as diagnostic tools.

Since the parasite surface must be the primary site for immune recognition a major part of the work has been directed at this compartment of the parasite. For protection, absolute specificity is not necessarily a prerequisite. Any surface antigens which do exhibit limited cross-reactions with antibodies to other parasites, however, could also be useful as diagnostic tools.

The major problem in diagnosis is, of course, specificity. It is reasonable to suppose that a given parasite will contain some species-specific antigen determinants. Also, unquestionably present will be a large number of molecules necessary for basic catabolic and anabolic activities. These are likely to be structurally conserved and thus highly cross-reactive. The objective, then, is to define those non-cross-reactive molecules suitable for diagnosis.

As a preliminary screen, our strategy has been to define stage-specific antigens of the nematode worm *T. spiralis*. The basic assumption is that there are greater chances of encountering a species (or genus)-specific antigen in the stage-specific compartment than amongst non-stage-specific parasite components. The choice of the nematode *T. spiralis* was dictated by its easy availability as a laboratory model, and by the expectation that principles established in this system can be extended to filarial parasites, where material is much more restricted in supply. In addition, the paucity of parasite material requires radiochemical procedures for increased sensitivity of detection and economical use of antigens.

Stage specificity is not, of course, confined to surface antigens. An immunochemical study of the somatic and excretory-secretory compartments of nematodes confirms this point, and provides a rational start for the design of immunodiagnostic tools and an understanding of the pathological consequences of nematodiases.

**Materials and methods**

Maintenance of *T. spiralis* and all of the experimental procedures have been previously described (Clark et al., 1982; Parkhouse & Clark, 1983).

**Surface antigens.** A comparison of the SDS/polyacrylamide-gel electrophoresis profiles of surface antigens of all stages is presented in Fig. 1. Under reducing conditions the labeled components were resolved thus: infective larvae, 105, 90, 55 and 47 kDa (Fig. 1b); newborn larvae, 64, 58, 34 and 30 kDa (Fig. 1d); adult worms, 40, 33 and 20 kDa (Fig. 1f). The pattern was quite different on the non-reducing gels. Infective larvae yielded a continuum of molecular size approx. 100 kDa to >1000 kDa (Fig. 1a) in addition to the 55 and 105 kDa bands. Newborn larvae were resolved into components of 120, 60, 34 and 32 kDa (Fig. 1e) (Clark et al., 1982).

At first sight, the simplest conclusion would be that assembly of surface antigens into multichain molecules occurs by disulphide-bond linkages. This is not, however, so. Instead, molecular aggregates relatively resistant to dissociation by SDS are formed in a disulphide-bond dependent, rather than disulphide-bond linked manner. Thus the infective larva surface antigens formed a spectrum of aggregates from 50 kDa to >1000 kDa from subunits of 47 kDa and 90 kDa; in the adult worms a 60 kDa complex arose from interaction between dissimilar molecules of 40 kDa and 20 kDa; the newborn larvae components formed homologous dimers from a 58 kDa molecule. Aggregating molecules were adherent to lentil lectin-Sepharose and are therefore glycoproteins. The interactions observed were completely abolished by boiling in SDS/mercaptoethanol, but only partially destroyed by boiling in SDS/iodoacetamide. Based upon this, the association can be characterized as non-covalent, but disulphide-bond dependent.

The major conclusions are immediately obvious: the pattern for each stage is simple (two to four bands) and absolutely stage-specific. From the point of view of the host, each succeeding stage represents a different antigenic challenge at the level of the parasite’s surface.

An important finding in this respect was that all of these surface glycoproteins were precipitated by antibodies present in sera taken from chronically infected mice. By definition, then, since they are antigens, the surface glycoproteins can participate in the immunological dialogue between parasite and host. Like many differentiation antigens on mammalian cell surfaces, these nematode antigens are also, as indicated above, glycoproteins with hydrophobic properties, but in the example of *T. spiralis* the sugar moieties are not exposed on the parasite surface.

Of particular interest was the finding that a monoclonal
antibody reactive with the 64 kDa component and staining the surface of newborn larvae was able to protect against infection in vivo (Ortega-Pierres et al., 1984). The same monoclonal antibody also mediated killing by eosinophils in vitro, suggesting a protective role in vivo for anti-nematode surface antibodies acting together with granulocytes. The observation that a single monoclonal antibody was able to protect significantly against infection is also an encouraging step towards the possible use of nematode surface antigens as vaccines.

**Secreted and somatic antigens.** The same three stages, infective larvae, adults and newborn larvae, were labelled in vitro with methionine (Parkhouse & Clark, 1983). The profiles of secreted [35S]methionine-labelled antigens on SDS/polyacrylamide gels were relatively simple and are so completely different as to suggest that all secreted components are stage-specific (Fig. 2). Not all, however, were antigens, that is, recognized by antibodies present in serum from conveniently infected mice. The secreted components, therefore, can in principle be used either for the detection of antibodies or parasites. In the former case the radioisotope-labelled antigen would serve as the probe. Detection of parasite antigens is something of a contradiction in terms, since antigens will combine with corresponding antibodies and become difficult, if not impossible, to detect. More suitable for this purpose are parasite components which fail to elicit antibody formation in conventional hosts, but which can do so in appropriately chosen experimental animals. Such components should also be released by the parasite in vivo to allow analysis in accessible host body fluids. In the model presented above, then, one possibility would be to first experimentally prepare antibodies against purified, or partially purified, secretory components that are not precipitated by sera from normal parasite infections. At this stage the choice of adjuvant, immunization course and experimental animal could be crucial. The resultant antiserum would then be used in a conventional inhibition assay with homologous radioisotope-labelled secretions and polyclonal serum of the appropriate species. A major advantage of biosynthetically labelled secreted antigen is that it is not contaminated by debris and/or products of dying organisms. This is certainly not so when worms are maintained in vitro, in the necessary absence of proteins, and the supernatants are radioisotope-labelled with iodine.

Analysis of whole-worm somatic constituents was restricted to angiostrongylin A-binding glycoproteins in order to reduce the complexity of somatic antigens examined. The resulting two-dimensional (O'Farrell, 1975) glycoprotein maps were very different for each stage, indicating a large number of stage-specific glycoproteins. Although not totally unexpected, in view of the very different morphologies of the three stages, none the less the result does hold hope for the employment of such somatic internal glycoproteins in diagnostic procedures.

For example, a conventional approach in detecting antibodies to parasites is to use a saline (or phosphate-buffered saline)-soluble parasite extract. Our prejudice is that such an extract is enriched for conserved molecules involved in anabolic and catabolic ('housekeeping') activities. Consequently, its use would be expected to encounter problems due to cross-reactions between similar, and perhaps distantly related, parasites. The finding of a large number of stage-specific, somatic glycoproteins in *Trichinella* suggests an alternative approach for the preparation of parasite antigens for diagnostic use. In this, parasites would be homogenized in a detergent, of the type used for solubilization of cell membranes, for example sodium deoxycholate. The soluble fraction would then be passed over a lectin column for recovery of the glycoprotein fraction and this would be used as the antigen probe.