Journal of Nematology 31(2):212–223. 1999.

Meloidogyne incognita Surface Antigen Epitopes in Infected *Arabidopsis* Roots[1]

M. J. Gravato-Nobre,[2] M. A. McClure,[3] L. Dolan,[4] G. Calder,[4] K. G. Davies,[2] B. Mulligan,[5] K. Evans,[2] and N. von Mende[2]

Abstract: Surface-coat epitopes of *Meloidogyne incognita* were detected in root tissues of *Arabidopsis thaliana* during migration and feeding site formation. A whole-mount root technique was used for immunolocalization of surface coat epitopes in *A. thaliana,* with the aid of a monoclonal antibody raised specifically against the outer surface of infective juveniles of *M. incognita.* The antibody, which was *Meloidogyne*-specific, recognized a fucosyl-bearing glycoprotein in the surface coat. During migration in host tissues the surface coat was shed, initially accumulating in the intercellular spaces next to the juvenile and later at cell junctions farther from the nematode. Upon induction of giant cell formation, the antibody bound to proximally located companion cells and sieve elements of the phloem.

Key words: antigen, antigenic mimicry, *Arabidopsis thaliana,* cell wall, host-parasite relationship, immunolocalization, *Meloidogyne incognita,* migration, nematode, surface coat, ultrastructure, whole-mount root technique.

The root-knot nematodes, *Meloidogyne* spp., are some of the least host-specific plant parasites. These obligate endoparasites rely completely on the formation of highly specialized feeding structures composed of several multinucleate giant cells (Endo and Wergin, 1973). Infective second-stage juveniles (J2) invade at the elongation zone of roots and migrate intercellularly through the cortex toward the root tip. They then reverse direction and move acropetally along a xylem pole until they reach the differentiation zone of the root, where they induce formation of giant cells from developing parenchyma cells (Gravato-Nobre et al., 1995; Gravato-Nobre, 1996; Wyss and Grundler, 1992). The nematode then undergoes three molts, emerging from the final molt as an adult female that is immobile, except for movement of the head.

Comprehensive descriptions of tissue modification at the feeding sites have been made in studies of *Meloidogyne*-host relationships over the past 50 years (reviewed by Endo, 1987). Nevertheless, little is known about the mechanisms by which these nematodes modify root development or the role(s) of their surface antigens in parasitism. Secretions released from granules formed in the esophageal glands are thought to be responsible for the changes induced in the host cells (Hussey et al., 1994), and it has been hypothesized that the surface coat is involved in dynamic interactions with host tissues (Almond and Parkhouse, 1985). The body of *Meloidogyne* spp. is enclosed by an extracellular cuticle, which in turn is covered by an indistinct layer known as the surface coat or glycocalyx. The surface coat can be resolved only by transmission electron microscopy, where it appears as an osmophilic layer that stains with Ruthenium red due to its carbohydrate content (Blaxter et al., 1992; Spiegel and McClure, 1995). This secreted layer is a common feature in a wide range of nematodes, including the plant-parasitic *Anguina* spp. (Bird, 1988; Bird and Zuckerman, 1989; McClure and Spiegel, 1991), the animal parasites *Trichinella spiralis* (Murrell et al., 1983) and *Toxocara canis* (Page et al., 1992), and

Received for publication 20 November 1997.
[1] Supported in part by the Gulbenkian Foundation, Lisbon, Portugal; the Biotechnology and Biological Sciences Research Council of the United Kingdom; and Grant No. IS-1894-90R from the United States-Israel Binational Agricultural Research and Development Fund (BARD).
[2] Entomology and Nematology Department, IACR Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, UK. Present address of first author: Zeneca Agrochemicals, Discovery, Jealott's Hill Research Station, Bracknell, Berks. RG42 6ET, UK.
[3] Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.
[4] Department of Cell Biology, The John Innes Centre, Colney Lane, Norwich NR4 7UH, UK.
[5] Department of Life Science, University of Nottingham, Nottingham, NG7 2RD, UK.
E-mail: maria.gravato-nobre@aguk.zeneca.com, mcclure@ag.arizona.edu
This paper was edited by B.C. Hyman.

the free-living nematode *Caenorhabditis elegans* (Zuckerman et al., 1979).

An important feature of the surface coat is its lability. Many nematodes, including *Meloidogyne incognita,* can shed and regenerate their surface coat (Bird and Zuckerman, 1989; Lin and McClure, 1996; Maizels et al., 1983; Maizels et al., 1984; Philipp et al., 1980). It appears that the surface coat has many functions. Modulation of the surface coat may help counter host defense responses, or surface-coat residues attached to animal host cells may help the nematodes to avoid detection (Almond and Parkhouse, 1985; Blaxter et al., 1992). The surface coat also may have a passive role, such as that of a lubricant to aid the passage of the nematode through its environment (Bird, 1988). In addition, nematode surface molecules are likely to be involved in other processes such as the adhesion of nematode-parasitic microorganisms (Bird and Zuckerman, 1989; Davies and Danks, 1992; McClure and Spiegel, 1991).

Our interest was to study the distribution of *M. incognita* surface coat epitopes in roots of the model host, *Arabidopsis thaliana,* and to assess their putative roles in parasitism.

Materials and Methods

Nematode and plant material: Egg masses from *M. incognita* (Kofoid & White) Chitwood 1949, race 1, NCSU #78, were dissected from eggplant (*Solanum melongena* L. cv. Blackbell) roots and incubated in water. Freshly hatched J2 were surface-sterilized in 0.01% $HgCl_2$ for 2 minutes, washed four times in sterile distilled water, and pelleted by centrifugation at 1,000g. The pellet was transferred to 1 ml of 0.05% Gelrite (Sigma, Poole, UK) and the suspension used to inoculate cultures of *Arabidopsis thaliana* (L.) Heynh cv. Landsberg *erecta.* Seeds were surface-sterilized (Sijmons et al., 1991) and grown in 9 cm diam. petri dishes containing 20 ml of Gamborg's B5 basal medium with minimal organics (Sigma), 1.5% sucrose, and 0.8% agar (Gravato-Nobre et al., 1995). Plants were grown at 23 °C in a 16:8 hour light:dark regime. Two-week-old seedlings were inoculated with about 50 J2/plant.

Production of monoclonal antibody: A monoclonal antibody, specific for *Meloidogyne* surface coat, was produced at the University of Arizona, Tucson and named MISC (*M*eloidogyne *I*ncognita *S*urface *C*oat). Antigen consisting of 0.01 ml (about 25,000) of live *M. incognita* race 3 J2 was mixed with Freund's complete adjuvant and injected intraperitoneally into female BALB/c mice. Four injections with the same dose per injection followed at 7-day intervals, and a final injection of the same preparation was administered 34 days later. Three days after the last injection, spleen cells were collected and fused with murine myeloma cells from myeloma cell line Sp2/0-Ag14 (Shulman et al., 1978), following standard protocols (Oi and Herzenberg, 1980). Hybridoma cells were cultured in Dulbecco's minimal essential medium (Life Technologies, Grand Island, NY) containing 7% fetal calf serum. Hybridoma supernatants were screened for antibodies that reacted with the surface of the nematodes by incubating live J2 in the supernatant, followed by four rinses with PBS and incubation in FITC-goat anti-mouse antibody (Sigma, St. Louis, MO). Treated nematodes were examined by UV epifluorescence microscopy, and hybridoma cell lines producing antibodies that bound to the nematode surface were cloned.

Cryosectioning of nematodes: Cryosections of J2 of *M. incognita, M. arenaria, M. javanica, M. hapla, Globodera pallida, G. rostochiensis, Heterodera schachtii, Ditylenchus dipsaci, Panagrellus redivivus,* and *Caenorhabditis elegans* were prepared. The nematodes were embedded in Bright Cryo-M-Bed (Bright Instruments Company Ltd., UK) and rapidly frozen by immersion in isopentane at −80 °C. Sections of 10 µm were cut on a Bright cryostat microtome model OTF/AS (Bright Instruments, Huntingdon, UK), thaw-mounted on poly-lysine-coated slides (Sigma, Poole, UK), and fixed in cold acetone for 10 minutes at 4 °C. Cryosections were then treated with 0.1% (v/v) Triton X-100 (TX-100) in PBS for 30 minutes.

Western blotting: About 50,000 J2 of *M. incognita* were homogenized in a microcentrifuge tube containing extraction buffer (10

mM Tris-HCl, pH 7.0; 25 mM sodium deoxycholate [NaDOC]; 5 mM ethylenediaminetetraacetic acid [EDTA]; 2 mM phenylmethylsulfonyl fluoride [PMSF]; 0.2 mM 1-tosylamide-2-phenyl-ethyl chloromethyl ketone [TPCK]; 0.2 mM N-tosyl-L-lysine chloromethyl ketone [TLCK], and 0.5 g sterile sand (40-100 mesh) at 4 °C. Forty micrograms of solubilized protein (Laemmli, 1970) were applied to a discontinuous SDS-polyacrylamide gel (3.9% acrylamide stacking gel, 10% acrylamide running gel) and separated electrophoretically (Laemmli, 1970). Samples of a prestained molecular weight marker, Sigma SDS-7B (Sigma, Poole, UK), were run on the same gel. Proteins were transferred to a 0.2-µm-pore nitrocellulose membrane (Sigma, Poole, UK) by electroblotting (Kyhse-Andersen, 1984) using a semi-dry unit (Hoefer Scientific Instruments, Newcastle-under-Lyme, UK). The membrane was blocked in 3% BSA/PBST for 4 hours, then incubated overnight at room temperature in the monoclonal antibody diluted 1:5 in the same blocking agent, followed by incubation in goat anti-mouse IgG alkaline phosphatase conjugate (Sigma, Poole, UK), diluted 1:500 in BSA/PBST. Alkaline phosphatase activity on the membrane was detected with buffered substrate tablets, Sigma Fast 5-Bromo-4-chloro-3-indolyl phosphate/Nitroblue tetrazolium (BCIP/NBT) (Sigma). Immunoblotting of surface-coat proteins was also accomplished by stripping the proteins from living J2 with a cationic detergent, cetyltrimethylammonium bromide (CTAB) (Lin and McClure, 1996), and blotting the electrophoretically separated extract as described above. To test the inhibition of MISC activity by fucose, the monoclonal was preincubated in 0.2M fucose in Tris buffer, pH 7.2, for 2 hours.

Tissue preparation for sectioning: Invaded root tips were excised and fixed in 0.2% glutaraldehyde and 2.5% paraformaldehyde in sodium cacodylate buffer (pH 7.2) for 4 hours at room temperature. After dehydration in a graded ethanol series, specimens were embedded in LR White acrylic resin (Agar Scientific, Stansted, UK). Sections of about 200 nm for light microscopy and 90-100 nm for transmission electron microscopy were cut with a glass knife on a Reichert Ultracut ultramicrotome (Reichert-Jung, Vienna, Austria). Details of tissue preparation have been described previously (Gravato-Nobre et al., 1995).

Whole mount root preparations: Ten-well slides (ICN Flow Lab, Thame, UK) were treated with 2% (v/v) APTES (3-Aminopropyltriethoxy silane, Sigma) in acetone for 10 seconds. After washing in acetone, slides were air-dried and, just prior to use, APTES was activated in 2.5% glutaraldehyde for 30 minutes. Root segments (about 2 mm long) invaded by J2 were fixed in a mixture of 4% formaldehyde and 0.1% glutaraldehyde for 1 hour at room temperature. After fixation, the segments were washed in a microtubule stabilizing buffer, MTSB (0.5 M PIPES, 1 M $MgSO_4$, 100 mM EDTA), mounted onto APTES coated slides, and allowed to dry for 20 minutes. Before immunolabeling, the root segments were treated with a mixture of 2% driselase (a mixture of laminarinase, xylanase, and cellulase; Sigma) and 2% cellulase (Sigma) for 7 minutes at room temperature.

Immunofluorescence labeling of resin-embedded and cryo-sections: The method for the immunolabeling of thin sections was described previously (Gravato-Nobre et al., 1995). Briefly, sections were incubated in 0.1% sodium borohydrate for 10 minutes and rinsed with PBS. After a 1-hour pre-incubation in PBS containing 3% BSA, sections were treated with MISC (diluted 1:50 in the same blocking agent) for 2 hours and washed thoroughly in PBS. Secondary antibody, goat anti-mouse IgG (whole molecule) FITC conjugate (Sigma) diluted 1:40, was then applied for 50 minutes, followed by repeated washing in PBS. Four negative controls were prepared: (i) sections treated with the second antibody only, (ii) with an irrelevant monoclonal, (iii) with Sp2 tissue culture supernatant, or (iv) with the serum of a pre-immunized mouse.

Immunofluorescence labeling of whole mount roots: After enzyme digestion (see above) root segments attached to 10-well slides were washed in 0.05% Tween 20 in MTSB for 15

minutes and air-dried for 20 minutes. They were then washed several times in MTSB for 20 minutes and blocked in 5% BSA for 30 minutes, followed by incubation with MISC for 1 hour at 37 °C, extensive washing, and incubation in goat anti-mouse IgG-FITC (described above) at the same dilution. Preparations were mounted in Citifluor (Agar Scientific), and whole mounts were examined under both light and confocal microscopes. Light micrographs were recorded on film; digital images were recorded with the confocal microscope's imaging system.

Immunogold labeling: Ultra-thin sections (90–100 nm) were processed and stained according to the method described by Gravato-Nobre et al. (1995). The working dilutions used were 1:50 for the primary antibody, MISC, and 1:40 for the secondary antibody, goat anti-mouse IgG, 10 nm gold conjugate (Sigma). Negative controls were prepared with either the Sp2 tissue culture supernatant or MISC inhibited by mixing the antibody with an equal volume of 0.2M L-fucose in 10 mM Tris-HCl buffer, pH 7.2, followed by incubation at 22 °C for 2 hours prior to use.

Microscopy: Thin sections were studied with an Olympus BH2 microscope equipped for epifluorescence. A Nikon Microphot SA microscope equipped for epifluorescence and differential interference contrast was used for whole-mount observations. Confocal images were generated with a MRC 1000 confocal microscope (Bio-Rad, Hemel Hempstead, UK). Series of optical sections were taken along the z-axis through the labeled cells. Ultra-thin sections were examined on a JEOL 1200EX transmission electron microscope operated at 60 kV.

Results

Localization and characterization of MISC-reactive epitopes in the surface coat: The epicuticle of the J2 is covered by a thin amorphous surface coat (Fig. 1A). MISC-reactive epitopes were detected in this coat (Fig. 1D), particularly labeling the cuticle annulations and lateral incisures (Fig. 1C). On Western blots of J2 homogenates, MISC recognized two major components of approximately 250 kDa and 190 kDa and some minor components, primarily 45 kDa and 35 kDa (Fig. 2, lane 1). These four components also were detected in the excretory-secretory products (ES) from living J2 incubated in a non-ionic detergent, Triton X-100, but only traces were detected in Triton X-100 washes of adult females. On Western blots of proteins stripped from living J2 with CTAB, only a single band of approximately 250 kDa was detected (Fig. 2, lane 2). When MISC was incubated with fucose prior to immunolabeling, antibody binding was blocked to the two major bands but not to the two minor bands. Antibody MISC bound to the surface of all four major *Meloidogyne* species (*M. hapla, M. javanica, M. arenaria,* and *M. incognita*) but not to the surface of other groups of plant-parasitic and free-living nematodes, including *Globodera* spp., *Heterodera schachtii, Ditylenchus dipsaci,* and *Panagrellus redivivus* (data not presented).

Localization of MISC-reactive epitopes during invasion and migration: Traces of the nematode surface coat were left on the root as the J2 contacted the root surface (Fig. 1B) just before invasion. During migration (Fig. 3A) the surface coat was shed and deposited on root cell walls in contact with the nematode (Fig. 4A). This trail of surface coat (Fig. 3B) clearly indicated the route taken by the J2 from the point of invasion to the feeding site in the stele.

Localization of MISC-reactive epitopes at the onset of giant cell formation: As the J2 became sedentary and induced the formation of giant cells, about 32 hours after invasion, the distribution of the surface coat changed. In the apoplast in immunogold-labeled thin sections, epitopes were found on cell walls adjacent to the J2 and at cell wall junctions not in direct contact with the nematode (Fig. 4B), as well as appearing to accumulate around the head of the nematode and up to 10 μm from the head (Fig. 5A, small arrow). In addition to the apoplast, MISC-reactive epitopes were also detected in the phloem, which was associated with developing giant cells (Fig. 5A,B, large arrow). These epit-

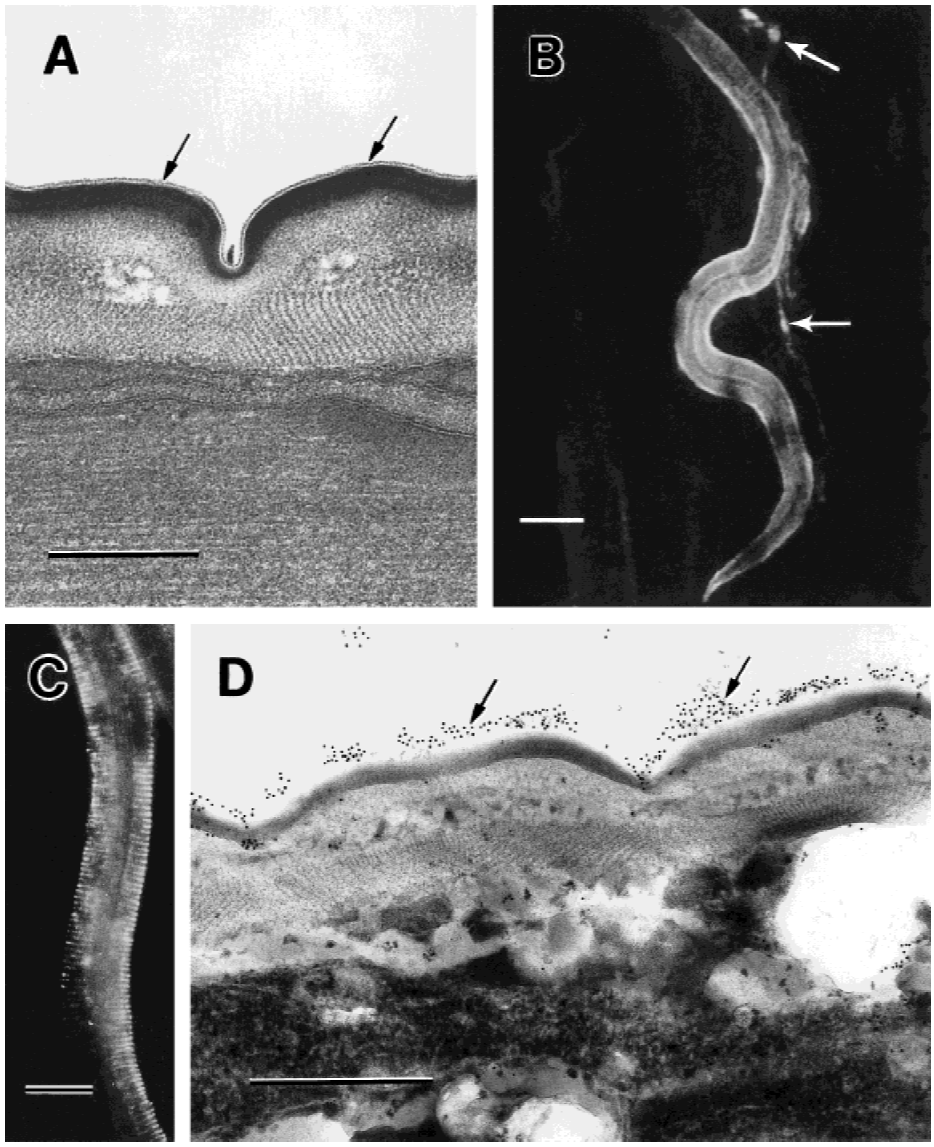

FIG. 1. Surface coat of *Meloidogyne incognita* second-stage juvenile (J2). A) Epicuticle is covered by a thin surface coat (arrow). Electron micrograph of a cross section through the cuticle, stained with uranyl acetate and lead citrate. Scale = 200 nm. B) Confocal microscope image of a J2 moving over a root and depositing surface-coat antigens (arrows) (labeled by the MISC antibody) on the root surface (black background) prior to invasion. Scale = 50 μm. C) Light microscope image on the detailed MISC labeling of the cuticle annulations and lateral incisures. Scale = 10 μm. D) Electron micrograph of a cross section through the cuticle. Surface coat was labeled with MISC and gold-conjugate secondary antibody (arrows). Scale = 500 nm.

opes were observed both in resin-embedded roots (Fig. 5A,C,D) and in whole-mount root preparations (Fig. 5B). At the early stages of giant cell formation, labeling around the nematode extended across the xylem parenchyma cells and to the companion cells of the phloem (Fig. 5D). These companion cells (Fig. 5C) were labeled at least 2 mm above the feeding site; sites farther from the nematode may have been labeled, but observations were hampered by lignification of the tissues. This pattern of labeling was observed in all 12 whole-mount root preparations that were examined. As the giant cells matured, both sieve tubes and companion cells of the phloem, positioned

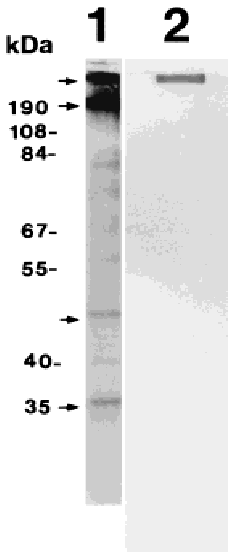

FIG. 2. Western blots of *Meloidogyne incognita* second-stage juvenile (J2) proteins probed with a monoclonal antibody (MISC). Lane 1, homogenate of J2. Two major proteins, *ca* 250 kDa and 190 kDa, were detected along with minor proteins of *ca* 45 kDa and 35 kDa (arrows). Lane 2, live J2 were washed with cetyltrimethylammonium bromide. A single protein of *ca* 250 kDa was detected.

next to the giant cells, were specifically labeled by MISC, but xylem cells in this region were not. Antibody binding was blocked when MISC was incubated with fucose prior to application (not shown). Labeling patterns during the infection process are summarized in Fig. 6.

Little background labeling was detected in uninfected roots probed with MISC and in infected roots treated with Sp2 cell line tissue culture supernatant. Some reactivity was detected in nematodes incubated in preimmune mouse serum, and minor background labeling occurred in plant tissues.

DISCUSSION

This is the first report of the detection of an antigenic epitope of a nematode surface coat at the nematode's feeding site within host plant tissues. The monoclonal antibody, MISC, raised to the *M. incognita* surface coat, was found to be *Meloidogyne*-specific. The specificity of MISC to fucosyl-bearing epitopes confirms observations made by Davies and Danks (1993), who detected fucose molecules in the surface coat of *M. incognita* with the lectin UEA.

Immunolocalization studies have shown that the nematode surface coat was shed during migration in *A. thaliana* roots. MISC-reactive epitopes were found on plant cell walls, which were in contact with the J2 as it passed through the cortex and vascular cylinder. Similar surface deposition in *Meloidogyne*-infected *Arabidopsis* roots was noted using a polyclonal antiserum specific to the cuticle of *Meloidogyne* spp. (Gravato-Nobre et al., 1995). Such antigen deposition during juvenile migration has been described for the animal-parasitic nematode *Toxocara canis,* when excretory-secretory antigens were detected in infected host tissues (Parsons et al., 1986). In our study, the wider distribution of MISC-reactive epitopes in the apoplast after accumulating around the nematode could have resulted from continuous production of the surface coat and the movement of these molecules apoplastically from the nematode and through cell corner junctions.

Secretions on the surfaces of nematodes may help the nematode evade host defense mechanisms by acting as a ''smokescreen'' (Blaxter et al., 1992) that masks the presence of the nematode. The suppression of the host immune response to *T. spiralis* is associated with the release of material during migration, thus ensuring the survival of the nematode (Almond and Parkhouse, 1985). On the other hand, surface molecules may also function as simple protecting and lubricating agents (Bird, 1988) or cementing substances (Endo and Wyss, 1992).

While the labeling during J2 migration was probably due to an epitope emanating from the nematode, the origin of the material found basipetally in association with the phloem remains undetermined. Similar cross-reactivity was observed by Bird and Wilson (1994), who showed that anti-phloem antibodies co-labeled the surface of adult *Meloidogyne* species. *Meloidogyne* surface coat molecules appear to share a fucosyl-bearing epitope with the phloem elements of infected roots. Because the binding of MISC

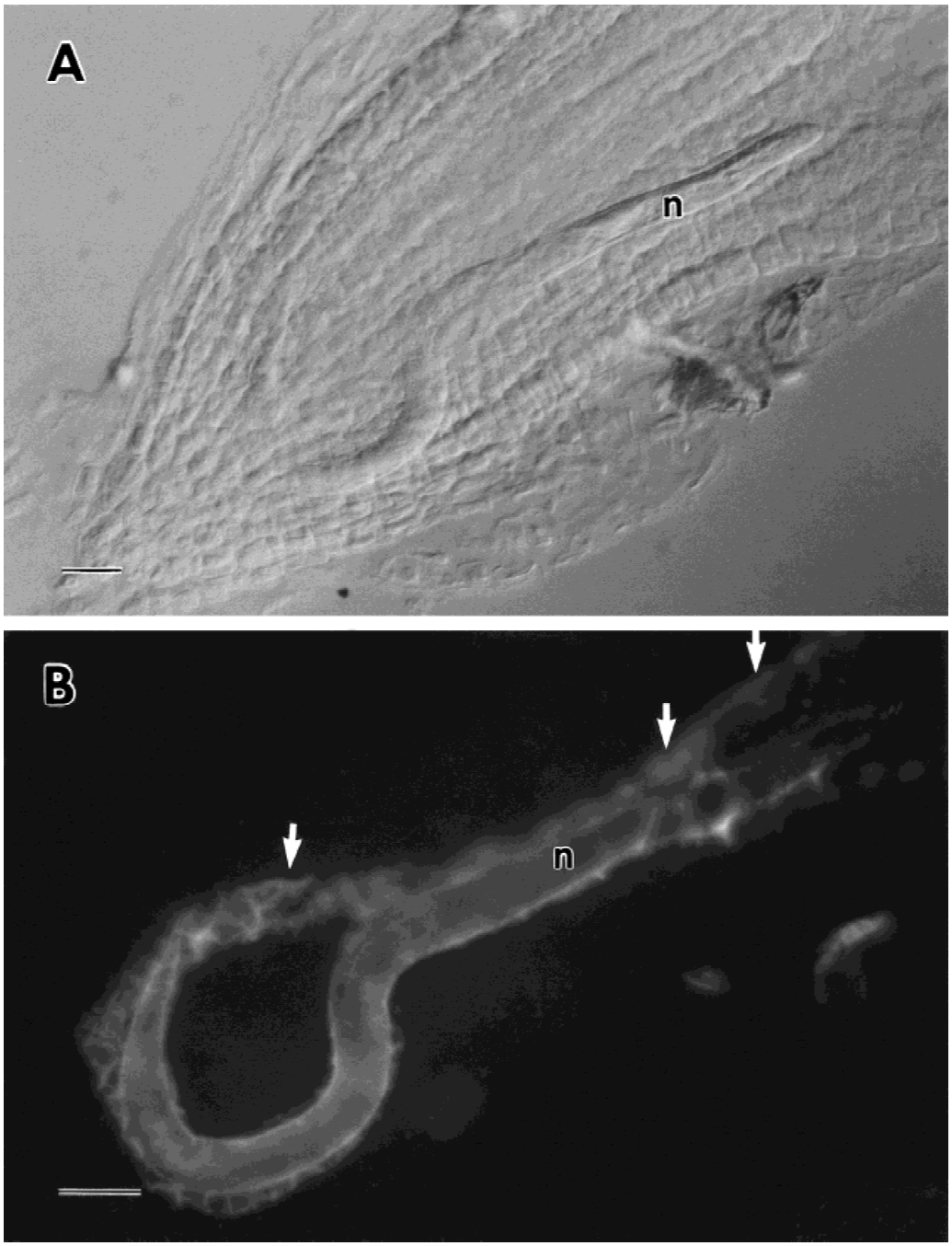

FIG. 3. Surface coat of *Meloidogyne incognita* second-stage juvenile (J2) during migration in *Arabidopsis thaliana* root near the meristem where the J2 reversed migratory direction. Root was probed with a monoclonal antibody (MISC) to the J2 surface coat and FITC-conjugated secondary antibody. A) Bright field image. B) Epifluorescence image. Arrows, deposits of J2 surface molecules along the nematode's migratory track. n, nematode. Scales = 10 µm.

to uninfected roots was not specific, we conclude that the fucosyl epitope identified by MISC in infected tissues is associated with parasitism by *M. incognita*.

Antibodies are epitope- but not necessarily antigen-specific, so the labeled molecules in the phloem could be of either plant or nematode origin. If they are of plant origin

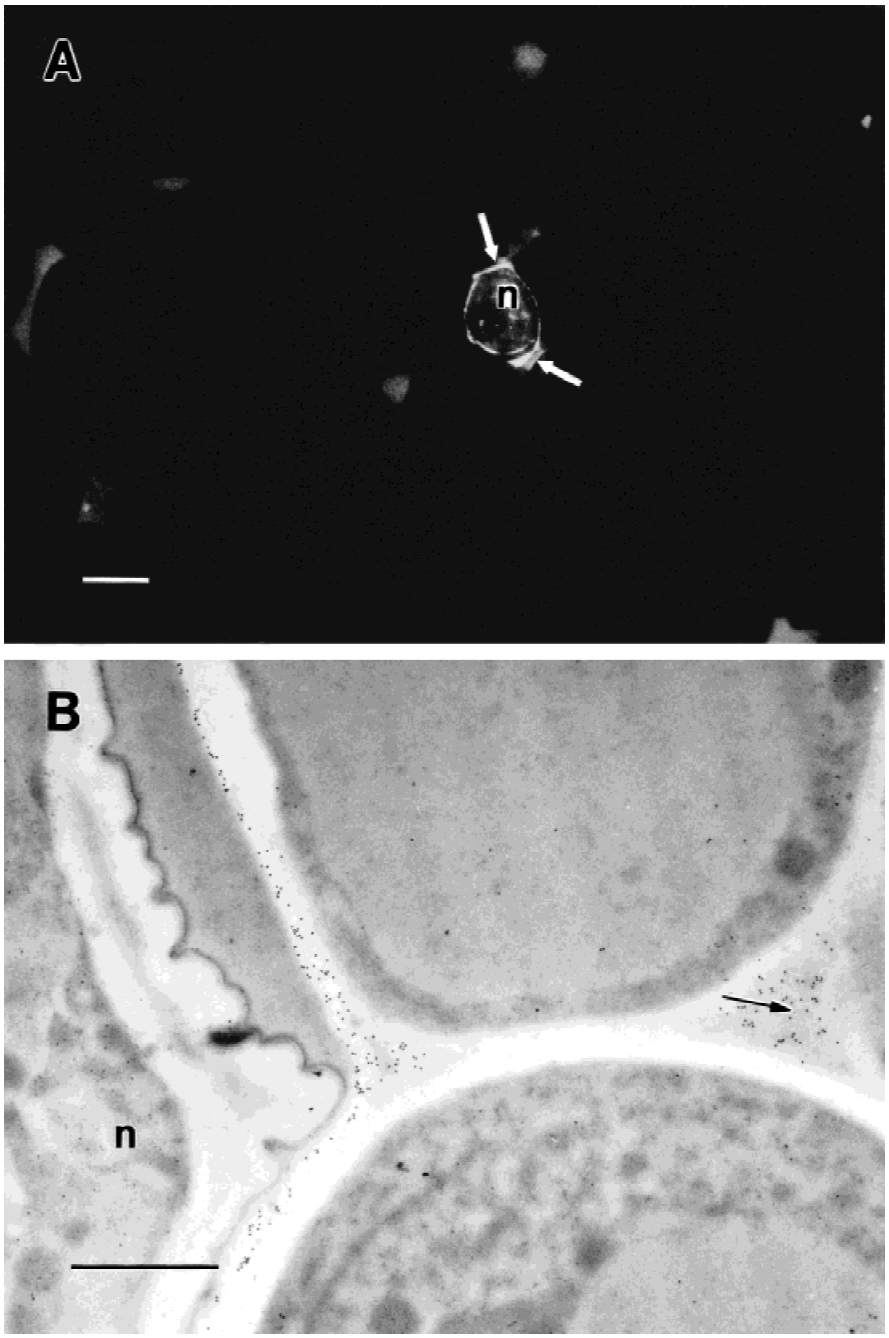

FIG. 4. Distribution of the surface coat of *Meloidogyne incognita* second-stage juvenile (J2) within the vascular cylinder of *Arabidopsis thaliana* roots. A) Surface coat material accumulated at the interface between the migrating J2 and the root cells (arrows). Epifluorescence of a root cross section, probed with MISC and FITC-conjugated secondary antibody. Scale = 10 µm. B) Surface coat material is labeled along the cell wall adjacent to the nematode and at three-way cell junctions away from the nematode (arrow). Electron micrograph of a root cross section at the onset of giant cell formation, probed with MISC and gold-conjugated secondary antibody. n, nematode. Scale = 1 µm.

they could be either part of the cell wall or components of the phloem exudate. Xyloglucan, a component of the cell wall, is a major hemicellulose with fucose present in its side chains (Masuda et al., 1989). As MISC labeling in uninfected roots was not

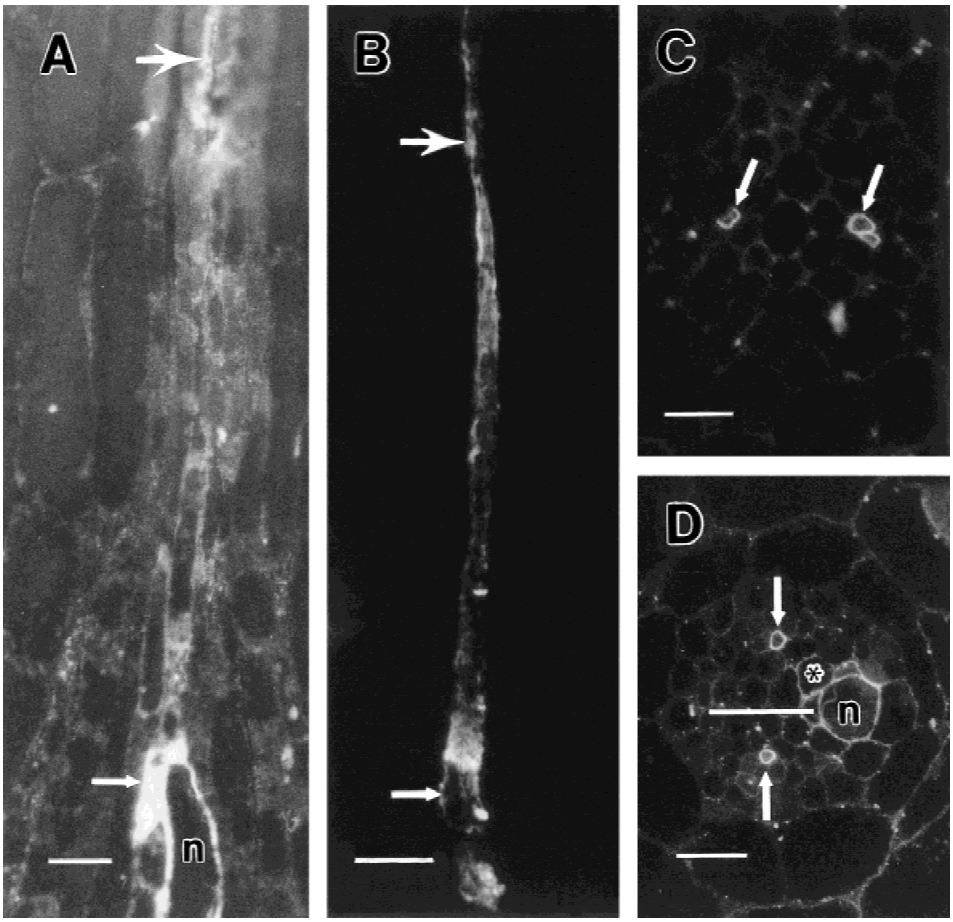

FIG. 5. Localization of MISC-reactive epitopes at the onset of giant cell formation by *Meloidogyne incognita* on *Arabidopsis thaliana* roots. A) The nematode's surface, accumulated surface coat (small arrow), and phloem cells above the nematode's feeding site (large arrow) are labeled. Epifluorescence image of a longitudinal section of a resin-embedded root, probed with MISC and FITC-conjugated secondary antibody. Scale = 10 μm. B) A file of phloem cells (large arrow) basipetally to the J2 (small arrow) were specifically labeled. Confocal image of a whole-root mount, probed with MISC and FITC-conjugated secondary antibody. Scale = 20 μm. C) MISC reactivity was detected in phloem companion cells (arrows). Epifluorescence image of a cross section through a resin-embedded root, basipetally to the feeding site. Scale = 10 μm. D) The nematode surface, xylem parenchyma cells (near asterisk), and phloem companion cells (arrows) were labeled. Epifluorescence image of a cross section through a resin-embedded root through the region of the nematode. Horizontal line, xylem axis; n, nematode. Scale = 10 μm.

strong, it is probable that these xyloglucan fucosyl-moieties were not expressed, were inaccessible, or were non-reactive to the antibody. In infected roots, these cell wall epitopes could have been made accessible for MISC binding as a result of parasitism by *Meloidogyne*. The epitopes may be present in the phloem sap and involved in bi- or unidirectional flow. A source-sink translocation would resemble that of carboxyfluorescein (Dorhout et al., 1993) and photosynthates (McClure, 1977) in the phloem of *Meloidogyne*-infected tomato roots. The alternative hypothesis is that the epitopes detected within the phloem are of nematode origin. If this is the case, it could be surmised that the secreted antigen is involved in antigenic mimicry and (or) signaling, and a major role for the surface molecules must be advanced. The esophageal glands, the excretory-secretory system (Choy et al., 1991; McLaren et al., 1987) or the epidermis (hypodermis) may all be sources of the products that accumulate on the surface of

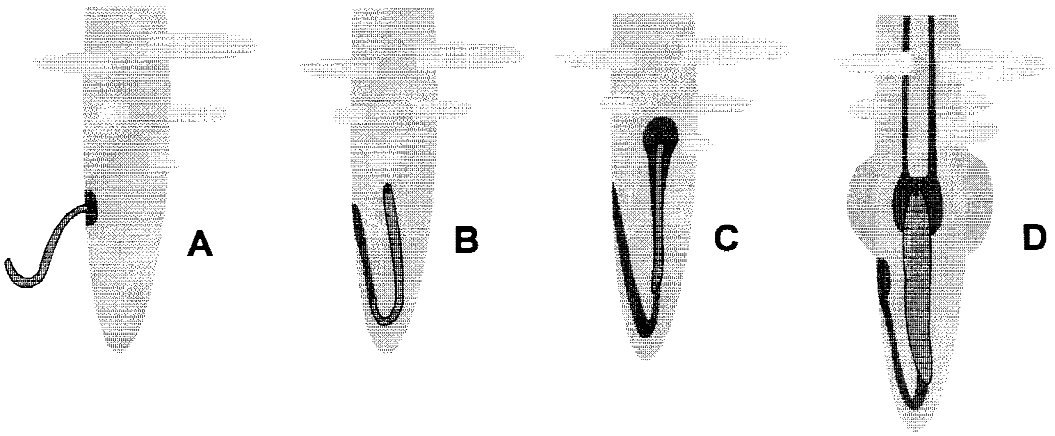

FIG. 6. Summary of the distribution of MISC labeling during early stages of infection of *Arabidopsis thaliana* by *Meloidogyne incognita*. A) Invasion: the second-stage juvenile (J2) invades the region of root tip elongation, depositing surface coat material on the root surface. B) Migration: the migrating J2 turns in the tip, shedding its surface coat as it passes between cells. C) Sedentary phase: accumulation of the surface coat around the head of the nematode and apoplastic movement of the material into the surrounding plant tissue. D) Giant cell initiation: additional labeling of the phloem.

nematodes (Bird, 1984; Wright, 1987), yet it is not known which component is responsible for induction of host responses. Mimicry of host antigens as a means of avoiding surface recognition has been described in various animals and plants infected with such diverse microorganisms as mycoplasms, bacteria, protozoa, fungi, and helminths (DeVay and Adler, 1976; DeVay et al., 1967; Chakraborty, 1988). Surface components of *Trichinella spiralis*, a mammal parasite, have been associated with the host nurse cells (Despommier et al., 1990; Kehayov et al., 1991; Lee et al., 1991; Vassilatis et al., 1992). Antigen sharing between *M. incognita* and the root tips of soybean and cotton was demonstrated more than two decades ago, but its role in the host-parasite interaction was not established (McClure et al., 1973).

In summary, we have shown that a fucosyl-bearing epitope, exposed on the surface of *M. incognita* J2, is shed during migration in host tissues and is specifically associated with infected root tissues after giant cell initiation. Further investigations will be required to determine whether such molecules are involved in the active evasion of host plant immune responses or whether they have a primary role in *Meloidogyne* parasitism. Generally, with increasing knowledge it seems increasingly clear that the surface coat plays a complex role in host-parasite relationships.

LITERATURE CITED

Almond, N. M., and R. M. E. Parkhouse. 1985. Nematode antigens. Current Topics in Microbiology and Immunology 120:173–203.

Bird, A. F. 1984. Nematoda. Pp. 212–233 *in* J. Bereiter-Hahn, A. G. Matoltsy, and K. S. Richards, eds. Biology of the integument, invertebrates. Berlin: Springer-Verlag.

Bird, A. F. 1988. Cuticle printing of nematodes. International Journal for Parasitology 18:869–871.

Bird, D. M., and M. A. Wilson. 1994. Molecular and cellular dissection of giant cell function. Pp. 181–195 *in* F. Lamberti, C. De Giorgi, and D. McK. Bird, eds. Advances in molecular plant nematology. New York: Plenum Press.

Bird, A. F., and B. M. Zuckerman. 1989. Studies on the surface coat (glycocalyx) of the dauer larva of *Anguina agrostis*. International Journal for Parasitology 19: 235–240.

Blaxter, M. L., A. P. Page, W. Rudin, and R. M. Maizels. 1992. Nematode surface coats: Actively evading immunity. Parasitology Today 8:243–247.

Chakraborty, B. M. 1988. Antigenic disparity. Pp. 477–484 *in* R. S. Singh, W. M. Hess, and D. J. Weber, eds. Experimental and conceptual plant pathology. Vol 3. Defense. New York: Gordon and Breach Science Publishers.

Choy, W. F., M. H. Ng, and P. L. Lim. 1991. *Trichinella spiralis:* Light microscope monoclonal antibody localization and immunochemical characterization of phos-

phorylcholine and other antigens in the muscle larva. Experimental Parasitology 73:172–183.

Davies, K. G., and C. Danks. 1992. Interspecific differences in the nematode surface coat between *Meloidogyne incognita* and *M. arenaria* related to the adhesion of the bacterium *Pasteuria penetrans*. Parasitology 105:457–480.

Davies, K. G., and C. Danks. 1993. Carbohydrate/protein interactions between the cuticle of infective juveniles of *Meloidogyne incognita* and spores of the obligate hyperparasite *Pasteuria penetrans*. Nematologica 39: 53–64.

Despommier, D. D., A. M. Gold, S. W. Buck, V. Capo, and D. Silberstein. 1990. *Trichinella spiralis:* Secreted antigen of infective L1 larva localizes to the cytoplasm and nucleoplasm of infected host cells. Experimental Parasitology 71:27–38.

DeVay, J. E., and H. E. Adler. 1976. Antigens common to hosts and parasites. Annual Review of Microbiology 30:147–168.

DeVay, J. E., W. C. Schnathorst, and M. S. Foda. 1967. Common antigens and host-parasite interactions. Pp. 313–328 *in* C. J. Mirocha, and I. Uritani, eds. The dynamic role of molecular constituents in plant-parasitic interaction. St. Paul, MN: American Phytopathological Society.

Dorhout, R., F. J. Gommers, and C. Kollöffel. 1993. Phloem transport of carboxyfluorescein through tomato roots infected with *Meloidogyne incognita*. Physiology and Molecular Plant Pathology 43:1–10.

Endo, B. Y. 1987. Histopathology and ultrastructure of crops invaded by certain sedentary endoparasitic nematodes. Pp. 196–210 *in* J. A. Veech and D. W. Dickson, eds. Vistas on nematology. Hyattsville, MD: Society of Nematologists.

Endo, B. Y., and W. P. Wergin. 1973. Ultrastructural investigation of clover roots during early stages of infection by the root-knot nematode, *Meloidogyne incognita*. Protoplasma 78:365–379.

Endo, B. Y., and U. Wyss. 1992. Ultrastructure of cuticular exudations in parasitic juvenile *Heterodera schachtii,* as related to cuticle structure. Protoplasma 166:67–77.

Gravato-Nobre, M. J. 1996. The importance of plant/nematode surface interactions in the infection of *Arabidopsis thaliana* by *Meloidogyne incognita*. Ph.D. thesis, University of Nottingham, Nottingham, UK.

Gravato-Nobre, M. J., N. von Mende, L. Dolan, K. P. Schmidt, K. Evans, and B. Mulligan. 1995. Immunolabeling of the cell surfaces of *Arabidopsis thaliana* roots following infection by *Meloidogyne incognita* (Nematoda). Journal of Experimental Botany 46:1711–1720.

Hussey, R. S., E. L. Davis, and C. Ray. 1994. *Meloidogyne* stylet secretions. Pp 233–249 *in* F. Lamberti, C. De Giorgi, and D. McK. Bird, eds. Molecular plant nematology. New York: Plenum Press.

Kehayov, I. R., S. D. Kyurkchiev, C. V. Tankov, and S. K. Komandarev. 1991. *Trichinella spiralis:* A 76-kDa excretory/secretory larval antigen identified by a monoclonal antibody. Experimental Parasitology 73: 150–160.

Kyhse-Andersen, J. 1984. Electroblotting of multiple gels: A simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide to nitrocellulose. Journal of Biochemical and Biophysiological Methods 10:203–209.

Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of the bacteriophage T4. Nature 227:680–685.

Lee, D. L., R. C. Ko, X. Y. Yi, and M. H. F. Yeung. 1991. *Trichinella spiralis:* Antigenic epitopes from the stichocytes detected in the hypertrophic nuclei and cytoplasm of the parasitized muscle fibre (nurse cell) of the host. Parasitology 102:117–123.

Lin, H-J., and M. A. McClure. 1996. Surface coat of *Meloidogyne incognita*. Journal of Nematology 28:216–224.

Maizels, R. M., M. Meghji, and B. M. Ogilvie. 1983. Restricted sets of parasite antigens from the surface of different stages and sexes of the nematode parasite *Nippostrongylus brasiliensis*. Immunology 48:107–121.

Maizels, R. M., D. de Savigny, and B. M. Ogilvie. 1984. Characterization of surface and excretory-secretory antigens of *Toxocara canis* infective larvae. Parasite Immunology 6:23–37.

Masuda Y., T. Hoson, R. Yamamoto, and M. Inouhe. 1989. Hormone-regulated modifications to cell wall polysaccharides: Relevance to cell separation. Pp 139–144 *in* D. J. Osborne and M. B. Jackson, eds. Cell separation in plants, NATO ASI Series, vol. H 35. Berlin: Springer-Verlag.

McClure, M. A. 1977. *Meloidogyne incognita:* A metabolic sink. Journal of Nematology 9:88–90.

McClure, M. A., I. Misaghi, and E. L. Nigh, Jr. 1973. Shared antigens of parasitic nematodes and host plants. Nature 244:306–307.

McClure, M. A., and Y. Spiegel. 1991. Role of the nematode surface coat in the adhesion of *Clavibacter* sp. to *Anguina funesta* and *Anguina tritici*. Parasitology 103: 421–427.

McLaren, D. J., G. Ortega-Pierres, and R. M. E. Parkhouse. 1987. *Trichinella spiralis:* Immunocytochemical localization of surface and intracellular antigens using monoclonal antibody probes. Parasitology 94:101–104.

Murrell, K. D., C. E. Graham, and M. McGreevy. 1983. *Strongyloides ratti* and *Trichinella spiralis:* Net charge of epicuticle. Experimental Parasitology 55:331–339.

Oi, V. T., and L. A. Herzenberg. 1980. Immunoglobulin-producing hybrid cell lines. Pp. 351–372 *in* B. B. Mishell and S. M. Shiigi, eds. Selected methods in cellular immunology. San Francisco: W. H. Freeman.

Page, A. P., A. J. Hamilton, and R. M. Maizels. 1992. *Toxocara canis:* Monoclonal antibodies to carbohydrate epitopes of secreted (TES) antigens localize to different secretion-related structures in infective larvae. Experimental Parasitology 75:56–71.

Parsons, J. C., D. D. Bowman, and R. B. Grieve. 1986. Tissue localization of excretory-secretory antigens of larval *Toxocara canis* in acute and chronic murine toxocariasis. American Journal of Tropical Medicine and Hygiene 35:974–981.

Philipp, M., R. M. E. Parkhouse, and B. M. Ogilvie. 1980. Changing proteins on the surface of a parasitic nematode. Nature 287:538–540.

Shulman, M., C. D. Wilde, and G. Köhler. 1978. A better cell line for making hybridomas secreting specific antibodies. Nature 276:269–270.

Sijmons, P. C., F. M. W. Grundler, N. von Mende, P. R. Burrows, and U. Wyss. 1991. *Arabidopsis thaliana* as a new model host for plant-parasitic nematodes. Plant Journal 1:245–254.

Spiegel, Y., and M. A. McClure. 1995. The surface coat of plant-parasitic nematodes: Chemical composition, origin, and biological role-a review. Journal of Nematology 27:127–134.

Vassilatis, D. K., D. Despommier, D. Misek, R. Polvere, A. M. Gold, and L. H. T. Van der Ploeg. 1992. Analysis of a 43-kDa glycoprotein from the intracellular parasitic nematode *Trichinella spiralis*. Journal of Biological Chemistry 267:18459–18465.

Wright, K. A. 1987. The nematode's cuticle-its surface and the epidermis: Function, homology, analogy—a current consensus. Journal of Parasitology 73: 1077–1083.

Wyss, U., and F. M. W. Grundler. 1992. Feeding behaviour of sedentary plant-parasitic nematodes. Netherlands Journal of Plant Pathology 98:165–173.

Zuckerman, B. M., I. Kahane, and S. Himmelhoch. 1979. *Caenorhabditis briggsae* and *C. elegans:* Partial characterization of cuticle surface carbohydrates. Experimental Parasitology 47:419–424.