

Effect of neuraminidase on the expression of the 3-fucosyl-N-acetyllactosamine antigen in human tissues

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SUMMARY The monoclonal antibody AGF4-48 detects the 3-fucosyl-N-acetyllactosamine carbohydrate structure and reacts with a variety of human tissues, as shown by an immunoperoxidase method on paraffin sections of formalin fixed material. The intensity and distribution of the immunoperoxidase reactivity were affected by treatment of sections with neuraminidase. In some sites, such as bone marrow, brain, breast, and pituitary, the reaction was more intense after neuraminidase treatment. In several organs, including kidney, oesophagus, pancreas, submandibular gland, and uterus, the reaction was more widespread after neuraminidase treatment. Other organs, such as liver, lung, lymph node, skin, and uterine tube, showed an altered distribution of reactivity with AGF4-48 after sections were treated with neuraminidase.

These results show that the 3-fucosyl-N-acetyllactosamine antigen is cryptic in many tissues. The antigen is masked to a variable extent in different tissues by neuraminic acid residues and can be revealed by the simple procedure of pretreating sections with neuraminidase. The value of using AGF4-48 antibody in combination with the enzyme neuraminidase to show accessible and cryptic antigen is that this reveals distinctive patterns of sialylation within various normal tissues. Whether similar patterns occur in disease will be of particular interest.

Little is known about the functions of particular sugar sequences within carbohydrate chains of cell surface and secreted glycoproteins or glycolipids. One aid to the understanding of this problem is to determine the precise distribution of specific carbohydrate structures within tissues. Some monoclonal antibodies to carbohydrate antigens can be used for such a study as the chemical structure of the determinant they recognise has been discovered. For instance, the monoclonal antibodies VEP8,¹ VEP9,¹ My-1,² and IG10³ detect the carbohydrate group 3-fucosyl-N-acetyllactosamine, which has the structure:



We have shown that another monoclonal antibody, AGF4-48, now known to react with 3-fucosyl-N-acetyllactosamine (T Feizi and HC Gooi, personal communication), can be used in immunohistological studies of formalin fixed, paraffin embedded tissues.⁴ We showed widespread distribution in human tissues of this antigenic determinant. Cells stained included proximal

tubules and thin limbs in the kidney, parietal cells in the stomach, a variety of other epithelial cells, astrocytes in the brain, and corticotrophs in the anterior pituitary.

Terminal galactose residues in carbohydrates are often sialylated by the addition of neuraminic acid residues.⁵ Furthermore, the 3-fucosyl-N-acetyllactosamine structure may not be detected in antibody binding studies because of steric hindrance by neuraminic acid residues.¹ Thus it was interesting to see whether removal of terminal neuraminic acid residues with the enzyme neuraminidase affected the immunohistological demonstration of 3-fucosyl-N-acetyllactosamine.

Material and methods

The mouse monoclonal antibody AFG4-48, raised against the human promyeloid cell line HL60, has been described previously.^{4,6} For immunohistology AGF4-48 hybridoma culture supernatant was used undiluted.

Fresh samples of normal human tissues, listed in the Tables, were obtained from surgical specimens

Table 1 *Effect of neuraminidase on reactivity of tissues with monoclonal antibody AGF4-48*

Tissues not stained by AGF4-48 even after neuraminidase treatment	
Adrenal, islets of Langerhans, parathyroid, thyroid	
Ovary, testis, trophoblast	
Bone, cartilage, connective tissues, all types of muscle, blood vessels, nerves	
Tissues stained by AGF4-48 with little effect of neuraminidase treatment	
Colon (a few basal cells)	
Duodenum (a few cells in ducts of Brunner's glands)	
Gastric mucosa (parietal cells, lumen of glands up to neck, luminal secretions)	
Prostate (a few epithelial cells)	
Tissues stained by AGF4-48 more strongly after neuraminidase treatment, but with unaltered distribution	
Anterior pituitary (corticotrophs)	
Bone marrow (myeloid series from promyelocyte stage)	
Brain (astrocytes)	
Breast (lactating lobules, milk, duct cells)	
Gall bladder (epithelial cells, mucus)	
Neutrophil polymorphonuclear leucocytes in all organs	

with the exception of samples of adrenal, brain, myocardium, parathyroid, skeletal muscle, and spinal cord, which were taken from necropsy samples. Tissues were fixed in formol-saline and embedded in paraffin. Sections were dewaxed, washed in tap water, and rinsed in veronal acetate buffer, pH 5.0. Control sections were covered with the same buffer solution while enzyme treated sections were covered with the buffer solution containing neuraminidase (from *Clostridium perfringens*, Sigma N 5631, lot 129C-8085-1). All sections were rocked in a covered chamber.

Preliminary experiments showed that the enzyme was effective at concentrations of 1 U/ml and 5 U/ml and that incubation times of 1–2 h at room temperature were as effective as 1 h at 37°C. Generally the enzyme was used at 1 U/ml for 1.5 h at room temperature.

After incubation, sections were washed well in tap water. Endogenous peroxidase was blocked with hydrogen peroxide in methanol and an indirect immunoperoxidase method was used as described previously using in the first stage AGF4-48 antibody and in the second stage peroxidase conjugated sheep IgG antibody to mouse IgG, IgA, and IgM (Serotec).⁴ AGF4-48 antibody adsorbed with HL60 cells was used as a control in the first stage.

Results

Sections incubated with AGF4-48 antibody that had been adsorbed with HL60 cells showed no staining by the peroxidase conjugated second antibody reagent whether or not the sections had been treated with neuraminidase. Control sections treated with veronal acetate buffer, pH 5.0, gave the same distribution of staining with AGF4-48 antibody as described previously.⁴

Neuraminidase treatment of sections affected the intensity and distribution of staining with AGF4-48 antibody in many tissues, although as noted in Table 1 the enzyme had little or no effect on the staining of some tissues. Tissues on which neuraminidase had no effect included some reactive with AGF4-48 antibody, such as gastric mucosa, and some unreactive with the antibody, such as thyroid. As noted in Table 1, the enzyme increased the intensity of staining of cytoplasmic granules of corticotrophs, astrocyte cell bodies and their processes, gall bladder epithelium and mucus in goblet cells, acini and milk in breast, and neutrophils, which showed peripheral accentuation and sometimes a granular intracytoplasmic reaction.

Table 2 lists those organs in which reactivity with

Table 2 *Tissues stained by monoclonal antibody AGF4-48 with altered distribution after neuraminidase treatment*

Tissue	Staining pattern on control sections	Staining pattern on neuraminidase treated sections
Liver	Very lightly in hepatocyte cytoplasm	Cell membrane of hepatocytes
Oesophagus	Superficial stratified squamous epithelium	All epithelium; submucous glands
Pancreas	A few acinar cells	All acinar cells
Submandibular gland	A few acinar and duct cells	All acinar cells; more duct cells
Kidney	Deep cortical proximal tubules; thin limbs of loop; superficial transitional epithelium, patchily	All proximal tubules; thin limbs; superficial transitional epithelium, strongly
Ureter, bladder	Superficial transitional epithelium, patchily	Superficial transitional epithelium, strongly
Cervix	Endocervical glands, patchily; superficial stratified squamous epithelium	All endocervical glands; mucus; all stratified squamous epithelium
Uterine tube	None	Scattered ciliated cells
Uterus	Basal endometrial glands	All parts of endometrial epithelium
Lymph node	None	Some large cells outside germinal centres
Thymus	Debris in Hassall's corpuscles	Stratified squamous epithelium; some large mononuclear cells
Tonsil	Superficial stratified squamous epithelium	All epithelium; some large cells infiltrating the crypt epithelium
Skin	None	Desmosomes, faintly; large cells infiltrating epidermis and dermis
Lung	None	Bronchial glands and epithelium; mucus
Nasal mucosa	None	Epithelial cells; mucus

AGF4-48 antibody was more widespread after enzyme treatment and also those organs in which reactivity became apparent only after neuraminidase treatment. Some different staining patterns observed on control and neuraminidase treated sections are also illustrated in Figs. 1–11.

The cell membrane of hepatocytes was not stained by AGF4-48 antibody in control sections but became strongly positive after neuraminidase treatment (Figs. 1 and 2). A similar but less striking change was seen in acinar cells of pancreas and submandibular gland. In kidney neuraminidase treatment affected the reactivity in that all proximal tubules were positive, not just those in the deep cortex (Figs. 3 and 4). In addition the enzyme increased the extent of staining of the transitional epithelium in the kidney, ureter, and bladder, and of stratified squamous epithelium in mucous membranes of the oesophagus, cervix, and tonsil.

Some glandular cells were either negative or incompletely positive in control sections but became uniformly positive after neuraminidase treatment; these included glands in the oesophagus and bronchus (Figs. 5 and 6) and endometrium. The cilia of some epithelial cells in the uterine tube became positive after enzyme treatment (Figs. 7 and 8).

In the skin there was only a slight increase in staining of the stratified squamous epithelium after neuraminidase treatment but Langerhans' cells in the epidermis and dermis became strongly positive, their cell bodies and dendritic processes being well shown (Figs. 9 and 10). Similar cells were seen in tonsillar crypt epithelium and in thymus and lymph node, in which they were probably interdigitating reticulum cells (Fig. 11).

Discussion

Several monoclonal antibodies have been shown to react with 3-fucosyl-N-acetyllactosamine. These include some antibodies like AGF4-48 raised against myeloid cells¹⁻³ and also some raised against various carcinomas.⁷⁻⁹ The monoclonal antibody to stage specific embryonic antigen I (SSEA-I) is against the same determinant.¹⁰ An immunohistological study with this antibody using unfixed sections gave results corresponding to those obtained in the present study when untreated sections were stained by AGF4-48 antibody, except that adrenal medulla and neurones were reported to be positive in the study of SSEA-I.¹¹

In this study we investigated whether the 3-fucosyl-N-acetyllactosamine antigen is cryptic and masked by neuraminic acid in various tissues by pre-treating sections with neuraminidase before staining with AGF4-48 antibody. Neuraminidase treatment

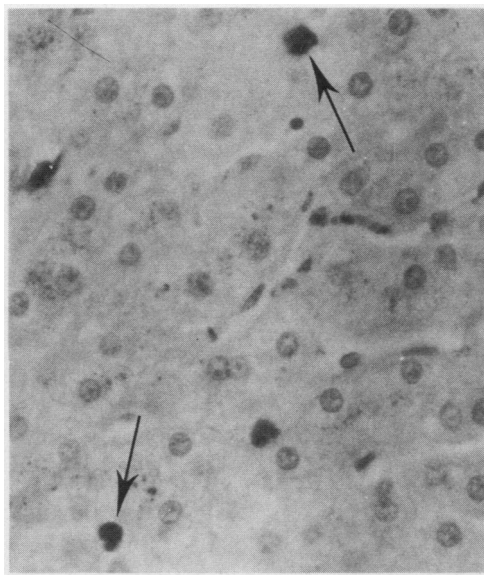


Fig. 1 A section of liver, incubated with buffer solution and then with monoclonal antibody AGF4-48. Neutrophil polymorphonuclear leucocytes stain strongly (arrowed), but staining of hepatocytes is virtually undetectable. Immunoperoxidase. $\times 480$.

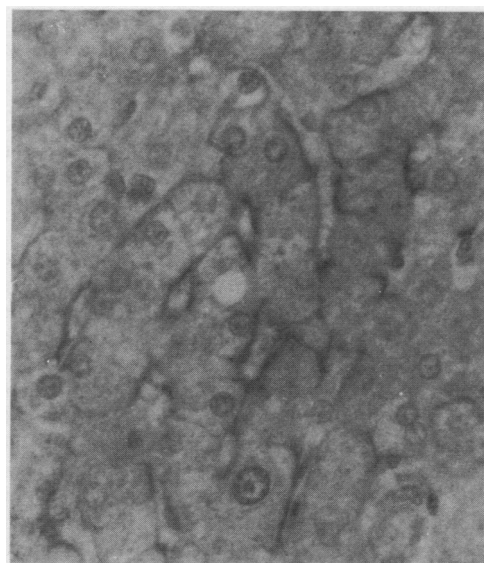


Fig. 2 An adjacent section of liver to that in Fig. 1, incubated with neuraminidase and then AGF4-48. The cell membrane of hepatocytes now stains strongly. Kupffer cells are negative. Immunoperoxidase. $\times 480$.

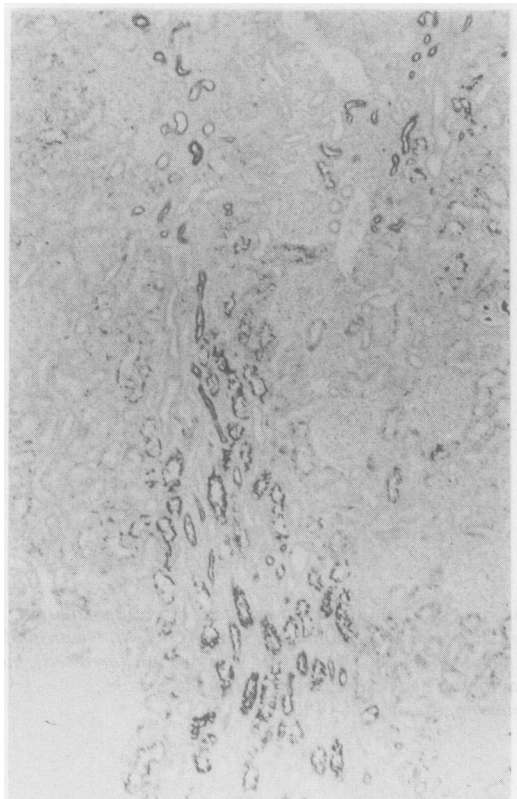


Fig. 3 *Renal cortex, buffer incubated. AGF4-48 stains the straight parts of proximal tubules and some juxtamedullary convoluted parts. Immunoperoxidase. $\times 48$.*

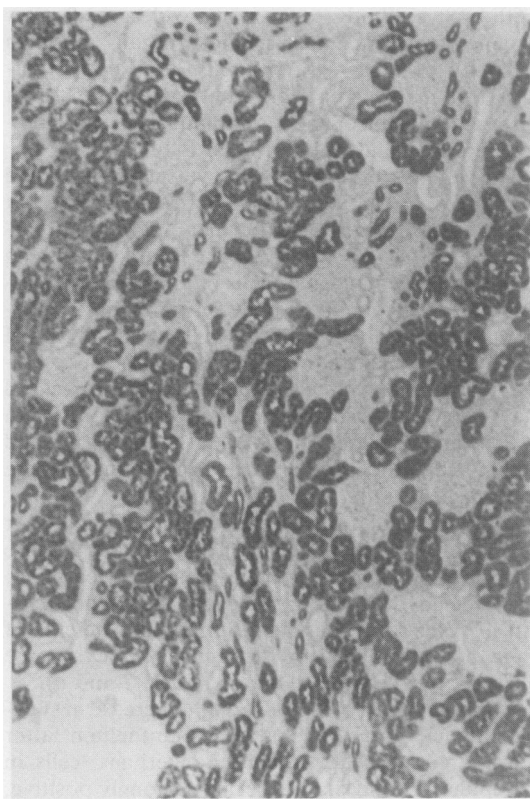


Fig. 4 *Renal cortex. An adjacent section to Fig. 3, neuraminidase incubated. All parts of all proximal tubules now stain strongly. Immunoperoxidase. $\times 48$.*

of tissue sections caused striking changes in the intensity and distribution of staining by AGF4-48 antibody. The results showed that 3-fucosyl-N-acetyllactosamine is obscured by neuraminic acid to a variable extent in different organs and, to a variable extent, even in different parts of the same organ—for example, the kidney.

The 3-fucosyl-N-acetyllactosamine antigen may be masked by neuraminic acid in two ways. One explanation of the effect of neuraminidase on the distribution of staining by AGF4-48 is that the enzyme is removing terminal neuraminic acid residues from 3-fucosyl-N-acetyllactosamine chains. Alternatively, neuraminidase is removing neuraminic acid residues from adjacent carbohydrate chains, which when sialylated obscure 3-fucosyl-N-acetyllactosamine by steric hindrance. A sialylated form of 3-fucosyl-N-acetyllactosamine does occur in human tissues. A glycolipid has been isolated from the kidney which has an N-acetyl-D-neuraminic acid residue on the 3-carbon

position of the galactose part of 3-fucosyl-N-acetyllactosamine.¹² In kidney AGF4-48 staining became strongly positive and more widespread after sections were treated with neuraminidase. The pronounced change may be related to removal of neuraminic acid residues from this glycolipid. Monoclonal antibodies which specifically recognise the sialylated form of 3-fucosyl-N-acetyllactosamine would be useful for investigating whether this antigen is masked by the addition of terminal neuraminic acid or by such residues on adjacent side chains. These are not yet available, although antibodies which react with other sialylated saccharides, such as the monoclonal antibodies Ca1,^{13,14} 19-9,¹⁵ and 4-2,¹⁶ have been reported.

The cryptic nature of the 3-fucosyl-N-acetyllactosamine saccharide in antibody binding studies illustrates a difficulty in using monoclonal antibodies to determine the precise occurrence of particular carbohydrate structures and thus

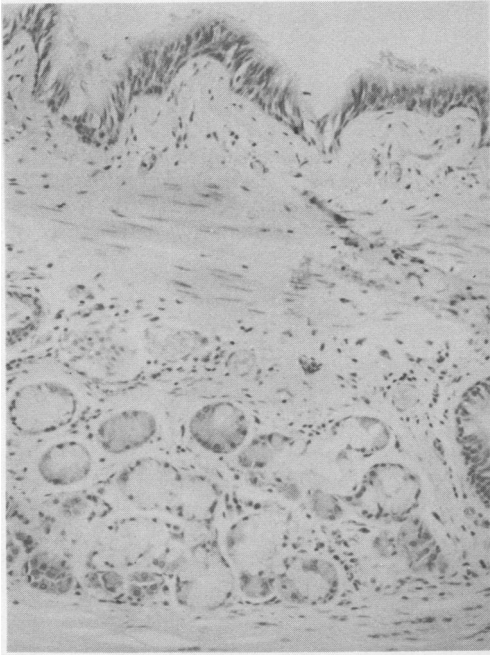


Fig. 5 Part of the wall of a bronchus, buffer incubated. Nothing reacts with AGF4-48. Immunoperoxidase. $\times 120$.

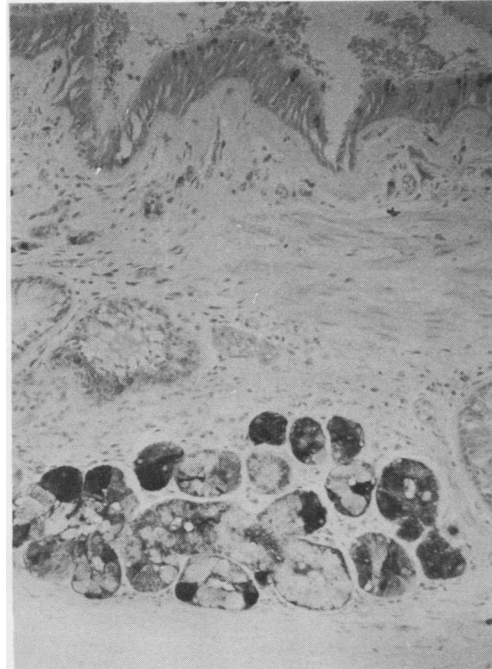


Fig. 6 An adjacent section of lung to that in Fig. 5, neuraminidase incubated. Bronchial glands and some ciliated epithelial cells react with AGF4-48. Immunoperoxidase. $\times 120$.

consideration of their significance as specific recognition structures. In many instances antibodies have been used to determine the tissue distribution of a particular antigen and suggest a correlation between the presence of the antigen and a physiological property of the cells. For example, monoclonal antibodies which are now known to identify the trans-

ferrin receptor initially described an antigen expressed in large amounts by rapidly proliferating cell populations.¹⁷⁻¹⁹ This study emphasises that monoclonal antibodies to carbohydrate antigens react only with accessible structures and that accessibility

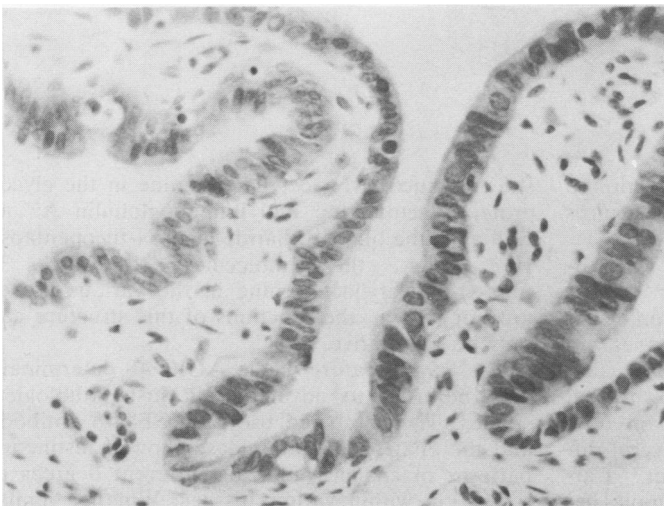


Fig. 7 Uterine tube, buffer incubated. The epithelium does not react with AGF4-48. Immunoperoxidase. $\times 300$.

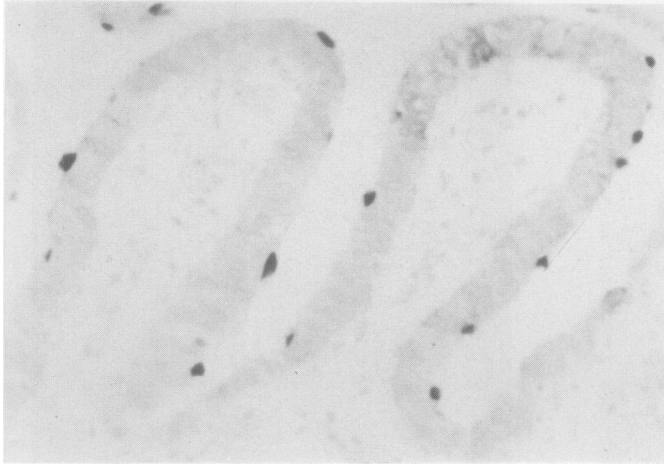


Fig. 8 An adjacent section of uterine tube to Fig. 7, neuraminidase incubated. Occasional ciliated epithelial cells react with AGF4-48. Immunoperoxidase. $\times 300$.

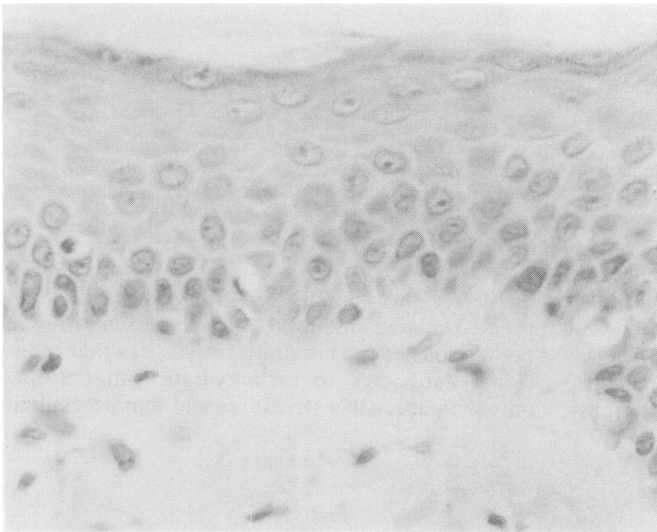


Fig. 9 Skin. After incubation with buffer no staining with AGF4-48 is detectable. Immunoperoxidase. $\times 480$.

in terms of antibody binding may bear no relation to the availability of particular structures as regards their physiological roles.

Consideration of the function of the 3-fucosyl-N-acetyllactosamine structure is further confused by the problem that the distribution of this antigenic determinant in various tissues does not necessarily indicate the presence of any particular glycolipid or glycoprotein. It is known that 3-fucosyl-N-acetyllactosamine can occur on both glycolipids and glycoproteins³ and that several different glycolipids can carry this determinant.^{2,8} Carbohydrate analysis of human milk, for example, has

shown 3-fucosyl-N-acetyllactosamine in the glycoproteins lactoferrin²⁰ and immunoglobulin A²⁰ as well as in the oligosaccharide lacto-N-fucopentaose III.²¹ As the molecules carrying 3-fucosyl-N-acetyllactosamine in the various tissues are not known, the functions of this structure can only be speculative.

The cryptic nature of the AGF4-48 determinant may be used to advantage in histopathological studies. The combined use of AGF4-48 antibody and the enzyme neuraminidase showed distinctive patterns of change reflecting different degrees of sialylation within various tissues. Whether similar

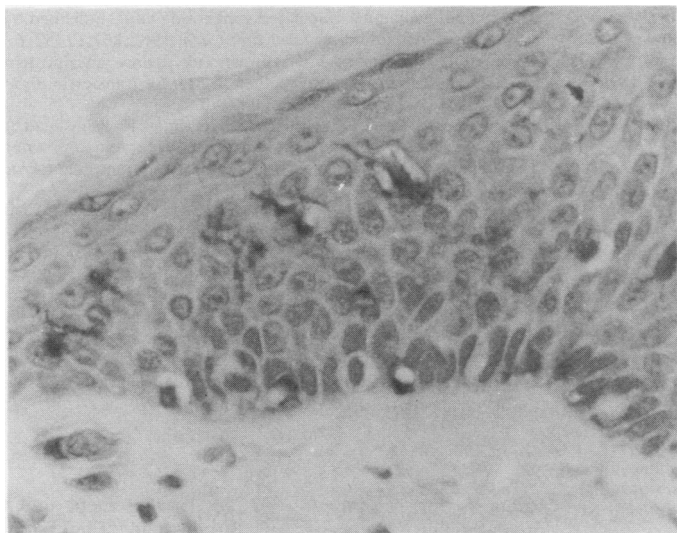


Fig. 10 Skin. An adjacent section to Fig. 9, treated with neuraminidase. AGF4-48 reacts with dendritic cells in the epidermis. Immunoperoxidase. $\times 480$.

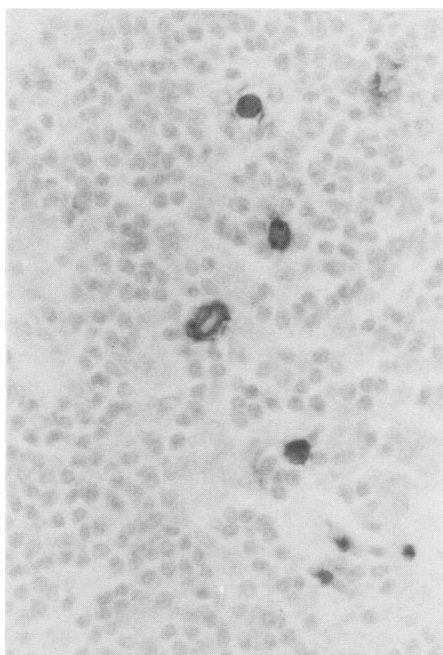


Fig. 11 A section of lymph node, incubated with neuraminidase. A few cells in interfollicular areas are stained by AGF4-48. Immunoperoxidase. $\times 480$.

changes occur to the distribution of AGF4-48 staining when sections of diseased tissues are treated with neuraminidase will be of particular interest.

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