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Patterns of lectin binding during mammalian neurogenesis

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ABSTRACT

Temporospatial changes in surface carbohydrates of neuroepithelial cells were analysed by means of lectin histochemistry in normal mouse embryos subsequent to closure of the neural tube. The lectins used were concanavalin A (con A), soybean (SBA), *Maclura pomifera* (MPA), peanut (PNA), wheatgerm (WGA), succinylated wheatgerm (sWGA) and *Limax flavus* (LFA). Although labelling was obtained with all of the lectins, the most striking temporospatial differences occurred with con A which in the early embryos (9–10 somites) labelled the basal and intercellular surfaces, but not the luminal surfaces of the neuroepithelial cells, whereas in the older embryos (26–30 somites), con A showed light luminal surface labelling. A midventral wedge of cells in the floor of the neural tube in the older embryos also exhibited more intense labelling with con A, WGA, and sWGA than with the other lectins. In addition, comparisons of lectin localisation were made between the closed neural tube in normal embryos and the open neural folds in the loop-tail (*Lp*) mutant mouse in which the neural tube fails to close. Although similar temporospatial patterns in lectin localisation occurred as in normal embryos, the retention of lectin labelling associated with rounded putative neural crest cells that remained sequestered in the apices of the open neural folds, along with an attenuation of the luminal reaction in the older abnormal embryos, suggest that during normal mammalian development closure of the spinal neural folds may be important for the timely exit of neural crest cells as well as for eliciting changes in the luminal surfaces of the neuroepithelial cells.

Key words: Mouse; neuroepithelium; neural tube closure; dysraphism; loop-tail mouse.

INTRODUCTION

Studies utilising lectins have yielded valuable information on cell surface carbohydrates during normal embryonic development, particularly before and during closure of the neural tube (Currie et al. 1984; Sato et al. 1986; Smits-van Prooije, 1986; Takahashi & Howes, 1986; Takahashi, 1988, 1992; Griffith & Wiley, 1989; Layer & Alber, 1990; Griffith & Sanders, 1991), as well as during relatively late stages of fetal development (DeGrauw & Liwnicz, 1986; Adam et al. 1993). However, it is unclear whether specific cell surfaces (e.g. basal vs luminal) of the mammalian neuroepithelium show distinctive temporospatial patterns of lectin binding which change during the intermediate phase of neurogenesis subsequent to closure of the neural folds, and whether these patterns serve as early markers to distinguish prospective pathways of differentiation. The current study thus analyses and compares the binding of a panel of 7 lectins during normal neurogenesis of the mouse: concanavalin A (conA), *Limax flavus* (LFA), *Maclura pomifera* (MPA), peanut (PNA), soybean (SBA), wheatgerm (WGA), and succinylated wheat germ (sWGA). Specific sites of interest are the basal, luminal and intercellular surfaces of the neuroepithelial cells, as well as distinctive features in the prospective roof plate and floor plate of the neural tube.

In addition, an important question is whether postclosure changes in the distribution of lectins are preprogrammed in the neuroepithelium before apposition and fusion of the neural folds, or whether the process of closure itself elicits or modulates subsequent changes in the pattern of cell differentiation, as reflected by lectin reactivity, particularly at the apices of the neural folds. Consequently, the effects of neural fold closure on the expression of lectin binding

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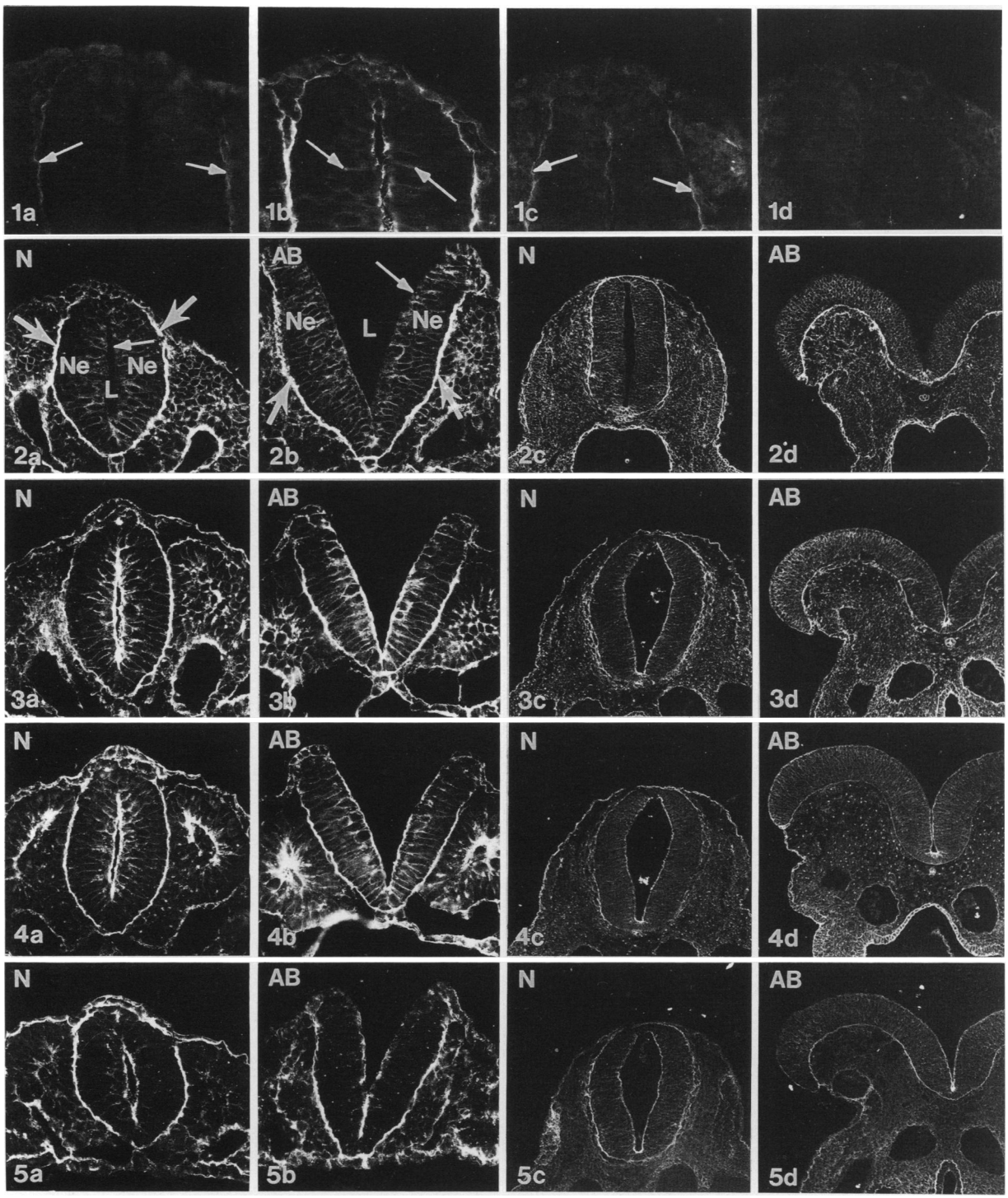


Fig. 1. Effects of neuraminidase (NA) pretreatment on lectin labelling. ×270. (*a*) SBA labelling (arrows) without NA. (*b*) SBA with NA. Note intensification of the reaction as well as visualisation of the intercellular reaction (arrows) not seen in (*a*). (*c*) LFA labelling (arrows) without NA. (*d*) LFA labelling is abolished with NA.

Figs 2–5. Cross sections of the closed neural tube in normal (N) and abnormal dysraphic (AB) embryos at 9–10 somites (*a*, *b*) and 26–30 somites (*c*, *d*) labelled with various lectins, as indicated. (*a*, *b*) ×170. (*c*, *d*) ×70. L, lumen; Ne, neuroepithelium.

Fig. 2. Con A. (*a*, *b*) The basement membrane (large arrows) and intercellular surfaces of the neuroepithelium are labelled, but the luminal surface (small arrow) is negative. (*c*, *d*) The luminal surface is faintly labelled.

Fig. 3. WGA. In normal embryos (*a*, *c*) the luminal surfaces are well labelled along with the basement membrane and intercellular surfaces. In the abnormal embryos the luminal surface is well labelled in 9–10 somite embryos (*b*) but poorly labelled in 26–30 somite embryos (*d*).

Fig. 4. sWGA. The pattern of labelling is similar to that with WGA (Fig. 3).

Fig. 5. MPA. The pattern of labelling is similar to that with WGA (Fig. 3).

Table. *Lectin labelling on cell surfaces (basal, luminal and intercellular) of the neuroepithelium in normal embryos at 9–10 somites and 26–30 somites*

	9–10 somites			26–30 somites		
Lectin (specificity)	Basal	Luminal	Inter	Basal	Luminal	Inter
Con A (Glc/Man)	++	−	+	++	+	+
+NA	++	−	+	++	+	+
SBA (GalNAc)	++	+	−	+	−	−
+NA	++	++	+	++	+	+
MPA (GalNAc)	++	++	+	++	++	+
+NA	++	++	+	++	++	+
PNA (GalNAc)	++	++	−	+	+	−
+NA	++	++	+	++	+	+
WGA (GlcNAc+SA)	++	++	+	++	++	+
+NA	++	++	+	++	++	+
sWGA (GlcNAc)	++	++	+	++	++	+
+NA	++	++	+	++	++	+
LFA (SA)	+	+	+	+	+	+
+NA	−	−	−	−	−	−

++, strong to moderate; +, light; −, not visible. +NA, with neuraminidase. Lectin specificity groups (see Damjanov, 1987): Glc/Man, glucose/mannose; GalNAc, N-acetylgalactosamine/galactose; GlcNAc+SA, N-acetylglucosamine+sialic acid; GlcNAc, N-acetylglucosamine; SA, sialic acid.

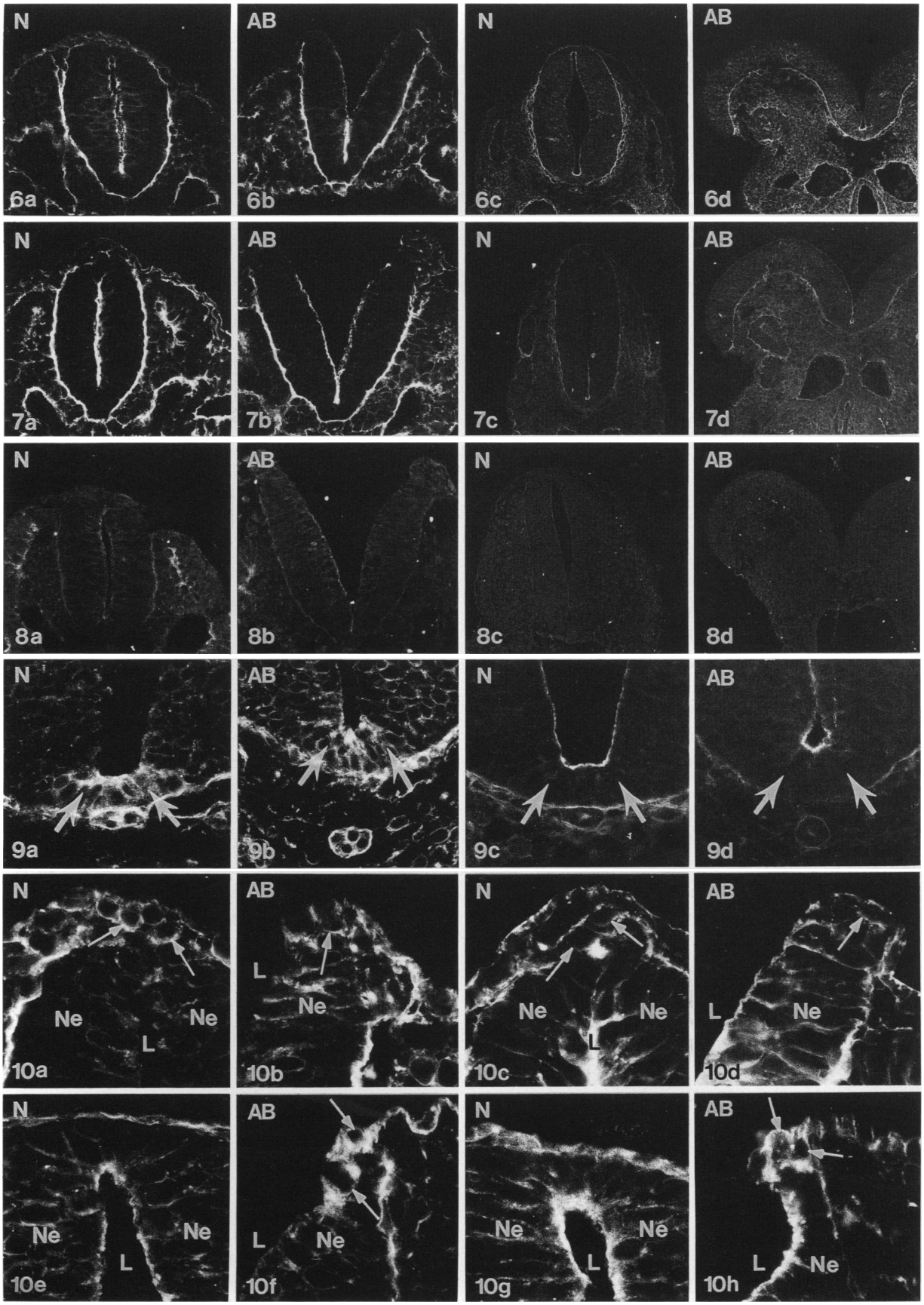
is analysed by comparing the distribution of these lectins in normal embryos subsequent to closure with that in comparable stages of abnormal dysraphic embryos of a mutant mouse (*Lp*) in which the neural folds fail to close (Stein & Rudin, 1953).

MATERIALS AND METHODS

Adult mice were maintained on a light–dark cycle (14 h light–10 h dark) under controlled temperature conditions. Normal (+/+) mice (A/St) were mated (+/+ × +/+) and abnormal loop-tail (*Lp*/+) mice on an A/St background (Strong & Hollander, 1949) were mated (*Lp*/+ × *Lp*/+); females were checked daily for vaginal plugs (day of plug = 0). Pregnant females were killed with light halothane anaesthesia followed by cervical dislocation. Embryos were removed in saline and staged according to somite number, counting all somites at stages prior to the appearance of otic placodes and counting postotic somites thereafter. Abnormal dysraphic (*Lp*/*Lp*) embryos obtained from *Lp*/+ × *Lp*/+ matings were easily identified by their lack of neural tube closure, and the abnormal (*Lp*/*Lp*) embryos along with normal (+/+) embryos (from +/+ × +/+ matings) ranging in age from 9–30 somites were age-matched. In addition, normal (?/+) littermates showing neural tube closure were selected and sampled from the *Lp*/+ × *Lp*/+ matings to provide additional controls for the abnormal *Lp*/*Lp* embryos. A total of 11 abnormal (*Lp*/*Lp*) and 15 normal (+/+; ?/+) embryos were obtained from 9 litters.

The embryos were fixed in Sainte-Marie's fixative for 1–2 h at 4 °C, lightly stained with methylene-azure blue for visibility, rinsed in 95% ethanol, and dehydrated to xylene. After embedment in Paraplast Plus, the blocks were stored at 4 °C. The blocks were sectioned at 8 μm, and the sections were placed on gelatin coated slides at 37 °C, after which the slides were stored at 4 °C.

After deparaffinisation and rehydration to phosphate buffered saline (PBS), the sections were exposed to drops of one of the following lectin-FITC conjugates (EY Laboratories, diluted in PBS) for 2 h at room temperature: concanavalin A (Con A, diluted 1:100), wheatgerm (WGA, diluted 1:100), succinylated wheatgerm (sWGA, diluted 1:50), *Maclura pomifera* (MPA, diluted 1:25), soybean (SBA, diluted 1:25), peanut (PNA, diluted 1:25), and *Limax flavus* (LFA, diluted 1:25). Slides were rinsed in 3 changes of PBS, coverslipped with Vectashield mounting medium (Vector Laboratories) and photographed using a fluorescence equipped Zeiss Photomicroscope III. Control slides for lectin-FITC specificity were processed similarly except the lectin-FITC was incubated with the appropriate competing sugar for 2 h before use. A significant decrease in lectin labelling following this incubation indicated specificity of binding. For neuraminidase (NA) pretreatment, some slides for each lectin were taken to Tris buffer (TB, 0.01 M,



Figs 6–8. Cross-sections of the closed neural tube in normal (N) and abnormal dysraphic (AB) embryos at 9–10 somites (*a*, *b*) and 26–30 somites (*c*, *d*) labelled with various lectins, as indicated. *a*, *b* ×170; *c*, *d* ×70.

Fig. 6. SBA. (*a*, *b*) With NA pretreatment the label of the luminal surface is moderate. (*c*, *d*) The luminal labelling is light, even with NA.

pH 6.3) and pretreated with drops of NA (EY Laboratories, from *Arthrobacter ureafaciens*, diluted to 0.5 U/ml of TB) at 37 °C for 3 h in a moist chamber. Controls for the NA sections were pretreated as above, but in TB only. Following rinses in TB and PBS the slides were exposed to lectin-FITC as described above.

RESULTS

All 7 lectins elicited a positive reaction associated with the neuroepithelium in early (9–10 somite) as well as late (26–30 somite) normal and abnormal embryos. Neuraminidase (NA) treatment tended to enhance the reaction for all of the lectins except LFA, either by intensifying the reaction or by unmasking a reaction that could not be visualised on some cell surfaces before NA treatment (Fig. 1). For LFA, however, NA abolished the reaction.

Normal embryos

The following account is based on an analysis of the temporospatial localisation of lectins in normal (+/+; ?/+) embryos without NA treatment (Figs 2–8) unless otherwise noted. A tabulation of these data is presented in the Table.

Along the basal surface of the neuroepithelial cells the neural basement membrane (BM) labelled at all stages with all 7 lectins, although the reaction obtained with SBA, PNA, and especially LFA was relatively light in the later stage embryos. However, this light basal labelling of SBA and PNA increased to a moderate reaction after NA treatment (Figs 6*a*, *c*, 7 *a*, *c*), whereas LFA was abolished.

The luminal surface of the neuroepithelial cells reacted with all of the lectins except con A, which was negative even after NA treatment in early stages. In later stages con A binding occurred but showed only a light reaction both with and without NA treatment. The luminal binding was light with SBA in early stages, but increased to a moderate reaction after NA treatment (Fig. 6*a*). However, in older embryos SBA failed to react with the luminal surface except for a faint label after NA. Although PNA showed a strong to moderate luminal reaction in early embryos, it declined to a light reaction in later stages and was unaffected by NA pretreatment.

A spatial difference was noted with respect to the luminal reaction in early embryos in which the midventral luminal surface showed a stronger SBA reaction than that along the lumen in lateral regions of the neural tube. In later embryos this difference became pronounced with all of the lectins, except LFA.

The intercellular surfaces of the neuroepithelial cells were outlined with all of the lectins except PNA and SBA. Compared with the basal and luminal reactions, however, the intercellular surfaces reacted relatively poorly and did not appear to be enhanced by NA, except for PNA and SBA which showed a light reaction after NA treatment.

A prominent difference occurred in the older embryos with respect to the neuroepithelial cells at the ventral midline of the neural tube. Here the cells were more heavily labelled with con A (Fig. 9*a*), WGA, and sWGA than were the cells in other sectors of the neural tube. In contrast, this increased label did not occur with the other lectins, even with NA pretreatment (Fig. 9*c*).

In the dorsum of the neural tube in early embryos, the reaction to the various lectins tended to be fragmented and irregular, due to the emigration of the rounded putative neural crest cells, which disrupted the basal laminar reaction as well (Fig. 10*a*, *c*). This disruption was not evident in the older embryos, where putative neural crest cells were rarely observed within the neuroepithelium (Fig. 10*e*, *g*).

Abnormal embryos

In the early 9–10 somite abnormal looptail mouse embryos, the neural folds had elevated but had not converged or fused, and by 29–30 somites the neural

Fig. 7. PNA. With NA pretreatment the pattern of labelling is similar to that with SBA + NA (Fig. 6).

Fig. 8. LFA. The labelling is poor, compared with the reaction of the other lectins.

Fig. 9. Lectin labelling in the midventral neuroepithelium (demarcated by arrows) in 26–30 somite normal (N) and abnormal dysraphic (AB) embryos. ×300. (*a*, *b*) Con A. Note intense midventral labelling. (*c*, *d*) PNA + NA. Note lack of increased midventral labelling, even with NA pretreatment.

Fig. 10. High magnification of lectin labelling of the dorsal neural tube in normal (N) embryos and in the comparable region of the open neural fold in abnormal dysraphic (AB) embryos. ×480. (*a–d*) 9–10 somite embryos; (*e–h*) 26–30 somite embryos. L, lumen; Ne, neuroepithelium. (*a*, *b*, *e*, *f*) Con A; (*c*, *d*, *g*, *h*) WGA. (*a–d*) Note discontinuities in the basement membrane along with labelled emigrating putative neural crest cells (arrows) emerging from the roof of the neuroepithelium. (*e*, *g*) The dorsum of the neural tube is intact and lacks emigrating putative neural crest cells. (*f*, *h*) Note presence of labelled rounded putative neural crest cells (arrows) within the neuroepithelium of the abnormal embryos.

folds had everted laterally. However, as in the normal embryos, all 7 lectins labelled the neuroepithelium (Figs 2–8), and NA pretreatment likewise tended to enhance the reaction for all lectins except for LFA, which was abolished. Although differences in labelling did not occur relative to the normal neuroepithelium with respect to localisation at the basal, luminal, and intercellular surfaces, in some instances the reaction tended to be more patchy and fainter along the luminal surface in abnormal embryos at 29–30 somite stages where the configuration of the abnormal neural folds produced a luminal convexity (Figs 3*d*, 4*d*, 5*d*). As in the normal embryos, the midventral wedge of neuroepithelial cells in abnormal 29–30 somite embryos was more highly reactive to the same group of lectins (Fig. 9*b*).

A prominent difference occurred between the older normal and abnormal embryos with respect to the dorsum of the neural tube (in normals) and the comparable portion of the open neural folds (in abnormals). Whereas the dorsum in normal embryos showed light lectin labelling, the apices of the folds in abnormal 26–30 somite embryos exhibited a strong reaction surrounding large rounded cells projecting from and associated with the junction between the neuroepithelium and surface epithelium (Fig. 10*f*, *h*). These rounded cells appeared to be putative neural crest cells that had not emigrated from the neuroepithelium and had retained not only basal and luminal labelling but, in many cases, a strong intercellular reaction as well.

DISCUSSION

Lectins serve as useful histochemical probes for studying the role of surface carbohydrates during normal development (Sanders, 1986; Damjanov, 1987; Griffith & Sanders, 1991). In the present study lectins have been used to determine whether differences could be detected among various cell surfaces during normal development of the neuroepithelium in the mouse subsequent to closure of the neural tube. Con A, which recognises glucose/mannose, showed the most distinctive temporal and spatial labelling in that the luminal surfaces of the neuroepithelial cells were negative in early (9–10 somite) embryos but became positive by the 26–30 somite stage. PNA, MPA, and particularly SBA, which recognises N-acetylgalactosamine/galactose, showed some subtle differences in intensity for basal, luminal, and intercellular surfaces at different ages, but our techniques did not enable us to quantify these differences. It is possible, however, for lectins which recognise the same monosaccharide to exhibit different affinities for different structures (Damjanov, 1987; Damjanov & Black, 1987). WGA, which recognises N-acetylglucosamine and sialic acid, and sWGA, which is specific for N-acetylglucosamine, labelled the various surfaces well, though subtle differences in intensity were also detected between the 2 lectins. LFA, which recognises sialic acid, showed overall the poorest reaction in our material for all surfaces at all stages; its abolition after NA treatment was consistent with previous studies (Takahashi, 1992).

It is not surprising that the basal surface of the neuroepithelium showed a relatively strong reaction with most of the lectins, since at the light microscopic level this surface includes the basement membrane (BM), as well as adjacent extracellular matrix (ECM) components recognised by lectins (Sanders, 1986). Whether or not the plasma membrane at the basal surface of neuroepithelial cells might show differential lectin reactivity awaits ultrastructural studies. Conversely, the relatively light labelling exhibited by the intercellular surfaces of neuroepithelial cells most likely reflects the absence of this association with BM and ECM.

The luminal surface of the normal neuroepithelium is of special interest because of the role it may play in mediating transport between the neuroepithelium and neural tube fluid in the lumen (Gato et al. 1993; Shepard et al. 1993). Thus the temporal acquisition of con A reactivity exhibited by this surface in our 26–30 somite embryos may reflect a physiological as well as structural maturation of the cells lining the lumen.

The intense reaction observed in the midline wedge of ventral neuroepithelial cells to Con A, WGA, and sWGA may reflect the distinctive structure of the prospective floor plate of the spinal cord as well as its role in dorsoventral differentiation of the neural tube (Smith, 1993). It has been shown that floor plate can induce motor neuron formation by diffusible inducing signal(s) (Placzek et al. 1991, 1993). During the early stages of neural tube development this wedge-shaped midventral region of the neural folds, designated as the median hinge point, has also been postulated as a possible stabilising or modulating force for neural fold elevation and fusion (Schoenwolf & Franks, 1984; Schoenwolf, 1985; Bush et al. 1990; Schoenwolf & Smith, 1990). The increased midline reactivity of the ventral neural tube may also reflect the underlying influence of the notochord, which in the present study likewise showed intense reactivity to the same lectins. Moreover, the notochord has been shown to play an important role in both contact-dependent and contact-independent differentiation of dorsoventral patterning

(Smith & Schoenwolf, 1989; Smith, 1993; Yamada et al. 1993).

The abnormal *Lp/Lp* embryos provide a useful tool with which to investigate the role which closure has on the subsequent differentiation of the normal neuroepithelium, since the neural folds remain open in this mutant, thereby not only depriving the neuroepithelial cells of a possible stimulus exerted by the process of apposition and fusion, but also exposing the neuroepithelium to continued contact with amniotic fluid. Although lectin reactivity of the various neuroepithelial cell surfaces in abnormal embryos was similar to that in normal embryos, two important differences occurred that may elucidate how closure normally affects differentiation.

In the older abnormal embryos, in which the luminal surface is convexly everted, the positive reaction to most of the lectins was more patchy and of lesser intensity than that in the normal embryos. It is possible that the abnormal configuration of the everted neural folds attenuated the strength of the reaction because the luminal surface of the neuroepithelium was convexly stretched. Previous ultrastructural studies have shown an overall flattening and reduction of villous surface projections from this surface in abnormal *Lp/Lp* embryos (Wilson & Finta, 1980*a*, *b*), which could result in the appearance of a less concentrated lectin reaction. However, it is also possible that failure of the neural folds to close deprived the neuroepithelial cells lining the 'lumen' of the appropriate stimulus to differentiate properly, as reflected by lectin reactivity. The chronic exposure of these cells to amniotic fluid, rather than neural tube fluid, may also have contributed to this difference in lectin reactivity.

A prominent difference was noted in the apical tips of the open neural folds of the older abnormal embryos. Here some putative neural crest cells with strong lectin reactivity were retained within the neuroepithelium, in contrast to the roof plate of the closed neural tube in normal embryos in which virtually all the putative neural crest cells appeared to have already vacated the neuroepithelium. Aberrant neural crest cells in the apical neural folds of this mutant have been observed ultrastructurally (Wilson & Wyatt, 1989). A delay in neural crest cell release has also been observed in *Sp* and Sp^d, two mutants which exhibit caudal spina bifida along with deficiencies in neural crest derivatives (Moase & Trasler, 1990), as well as in chick neural tube defects induced experimentally by means of antisense oligonucleotides (Nieto et al. 1994). Although the neural crest cells may be a primary and direct target of the gene in some mutants, this does not appear to be the case in *Lp*, where the elevation of neural folds is defective considerably earlier in development, and the folds do not even approach one another sufficiently for apposition and fusion to occur (Wilson & Wyatt, 1992).

During normal development of the mouse, virtually all the neural crest cells in the prosencephalic region and most in the midbrain area of the cranial neural folds exit from the neuroepithelium prior to fusion, whereas in the rhombencephalon some neural crest cells emerge before and after fusion (Innes, 1985; Nichols, 1987; Hoving et al. 1990). Thus in some cases the initial release of these cells does not seem to require neural fold fusion. In contrast, in the trunk neural folds the neural crest cells do not apparently emerge until after fusion (Erickson & Weston, 1983; Sternberg & Kimber, 1986*a*, *b*). Although the stimulus for emigration is at present not clear (Erickson & Perris, 1993), the results of the current study suggest that in the trunk region of the neural folds the timely departure of neural crest cells may be dependent on closure as a stimulus, as suggested by the sequestration of some of these cells associated with closure defects.

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