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Original Article

Expression and distribution of carbohydrate sequences in chick germ cells: a comparative study with lectins and the NC-1/HNK-1 monoclonal antibody

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ABSTRACT The expression of end-chain sugar residues and of oligosaccharidic sequences has been investigated in chick germ cells at critical stages during the migration, proliferation and sexual differentiation of these cells. Fluorescent lectins and indirect immunofluorescence studies using the NC-1/HNK-1 monoclonal antibody indicate a remarkable control of glycosylation during germ cell embryonal life. Besides a retained expression of glucose/mannose residues, it was found that α- and ß-galactose residues, N-acetyllactosamine and N-N' diacetylchitobiose sequences as well as the sulfated trisaccharidic NC-1 epitope were detectable in a stage-specific pattern. Present at a very high density in the cytoplasm and on the surface of the early germ cells at premigrative and migratory stages, the staining for these carbohydrate sequences gradually disappeared when the germ cells settled and proliferated in the developing gonadal primordia. The disaccharide Galß1→3 Gal NAc was exclusively detected in migrating PGCs. In sexualized gonads, acetyllactosamine and/or diacetylchitobiose were similarly reexpressed in both oogonia and spermatogonia. Spermatogonia displayed ß-galactose residues and a high immunoreactivity with the NC1 Mab, indicating modulations in PGC glycosylations related to the acquisition of sexual phenotypes. In addition NC-1 was found to be expressed in the somatic component of the undifferentiated gonad and in the testis interstitial gland.

KEY WORDS: *carbohydrate sequences, chick germ cells, lectins, anti-NC-1/HNK-1 monoclonal antibody*

Introduction

During the development of vertebrates, directional cell migrations involve a range of reciprocal specific interactions between migrating cells and their local microenvironment at defined stages. For instance in the developing nervous system, migrations and subsequent integration of cells in peripheral or central nervous structures involve modulated cooperations between CAMs, specialized membrane receptors and components of the extracellular matrices (ECMs) (Thiery *et al.*, 1985, 1990; Erickson, 1987; Duband *et al.*, 1988; Hammarback *et al.*, 1988; Halfter *et al.*, 1989; Stallcup *et al.*, 1989; Humphries and Yamada, 1990).

The avian primordial germ cells (PGCs), which are the precursors of the reproductive cells, are also migratory elements. However when compared to neural crest cells, PGCs display distinctive characteristics that could involve differences in their migratory behavior. In the germinal crescent, PGCs appear as scattered elements (about 150 cells in the chick at the primitive streak stage) constituting a very low cell density population. At stage 9 H.H. (Hamburger and Hamilton, 1951) most of them are progressively enclosed in the developing vascular network. It is still unclear whether PGCs are passively incorporated in the forming blood vessels or whether they actively enter vascular endothelia. As early as stage 15, an increasing proportion of germ cells can be observed in the capillaries of the future gonadal area caudal to the emergence of the omphalomesenteric arteries (Meyer, 1964; Fargeix *et al.*, 1981). Most of them have been passively carried to the trunk *via* the circulating blood (Fujimoto *et al.*, 1976a) as a consequence of the

Abbreviations used in this paper: Fuc, fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; Glc, glucose; Glc A, glucuronic acid; GlcNAc, N-acetylglucosamine; Man, mannose; Neu NAc, neuraminic acid; Mab, monoclonal antibody; NC-1, neural crest; HNK-1, human natural killer; CAM, cell adhesion molecule; SAM, substrate adhesion molecule.

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TABLE 1

LIST OF FLUORESCENT LECTINS AND MAB USED IN CGP BINDING

Taxonomic name	Abbreviation	Inhibitory sugar
Canavalia ensiformis	Con A	α D Man, α D Glc
Pisum sativum	PSA	" "
Lathyrus odoratus	LOA	" "
Lens culinaris	LCA*	" "
Limulus polyphemus	LPA*	Neu N Ac
Ulex europaeus	UEA-1*	Fuc α1 → 2 Gal
Laccaria amethystina I	LAF*	"
Bandeiraea simplicifolia	BS II*	D GlcNAc
	BS I-A4*	α D GalNAc
	BS I-B4	α D Gal
Glycine max	SBA(Soybean)*	D GalNAc
Sophora japonica	SJA*	ß D GalNAc
Helix pomatia	HPA	D GalNAc, α D GlcNAc, α D Gal
Phaseolus vulgaris	PHA (E+L)	Neu N Ac→ß D Gal 1→3
	phytohemagglutinin	GlcNAc 1→2 α D Man
Ricinus communis	RCA 120	ß D Gal
Arachis hypogea	PNA (peanut)	ß D Gal 1→3 Gal NAc
Polyporus sulfureus	PoSA	Galß 1→4 Glc, Galß 1→4 GlcNAc
Laccaria amethystina II	LAL	Galß 1→Glc, GalNAc
Lactarius torminosus	LTA	unknown
Erythrina cristagalli	ECA	Galß 1→4 GlcNAc, Galß 1→4 Glc
Datura stramonium	DSA	$(D\ GlcNAc)_2$, Galß 1→4 GlcNAc
Lycopersicon esculentum	LEA	" "
Solanum tuberosum	STA*	$(D\ GlcNAc)_3$
Triticum vulgaris	WGA (Wheat germ)	$(D\ GlcNAc)_2$, Neu NAc
Neural crest cell Mab	NC1(=HNK 1)	Sulfate 3Glc A ß1→3 Galß 1→4 Glc NAc

* Lectins which do not bind PGCs at any stage.

endomesodermal rearrangements involved in vasculogenesis, coelom organization and gut morphogenesis. Then a period of active interstitial migration starts during which PGCs leave the vessels, move through the dorsal mesentery ECM in a dorso-ventral direction, and then settle into the gonadal primordia (Dubois and Croisille, 1970; Swartz , 1982; Ando and Fujimoto, 1983; Didier *et al.*, 1983).

Although experimental evidence has been presented of a chemotactic attraction exerted by the gonadal epithelia upon PGCs (Dubois, 1966; Kuwana *et al.*, 1986) the molecular interactions involved in germ cell migration still remain poorly understood. The distribution of several ECM components (laminin, fibronectin, chondroitin sulfate proteoglycan, collagen IV) has been recently documented in the PGC migratory pathways (Urven *et al.*, 1989); however it has not been established whether any of these molecules govern PGC adhesion or locomotion.

In addition, cytological and biochemical characteristics of PGCs themselves may account for their migrative properties. Chick germ cells can be detected from the primitive streak stage owing to their high glycogen content (Meyer, 1964; Fujimoto *et al.*, 1976b; Fargeix *et al.*, 1981) or their phosphatase activity (Swartz, 1982). In the quail, the immunoreactivity of germ cells with various antibodies led to the identification of germ cell precursors at pregastrula stages, thus adding weight to arguments in favor of their epiblastic origin (Pardanaud *et al.*, 1987; Ginsburg *et al.*, 1989a). In the chick, a variety of carbohydrate antigens have been shown to be simultaneously expressed by germ cells and other embryonic cell types including some mammalian carcinoma cell lines (Thorpe *et al.*, 1988; Urven *et al.*, 1988; Loveless *et al.*, 1990). Prominent in PGCs are sialylated and non-sialylated forms of antigens belonging to the poly-N-acetyllactosamine series. This point is of special interest when the potential role of carbohydrates as recognition molecules during development is considered.

Developmentally regulated alterations in glycosidic determinants may occur during the differentiation of various cells including the mammalian germ cell line (Fox *et al.*, 1981; Sato and Muramatsu, 1986; Sato *et al.*, 1986; Fazel *et al.*, 1987; Muramatsu 1988 a, b; Kanai *et al.*, 1989). In the current work we describe a similar variability in the expression of certain carbohydrate epitopes in the avian germ cell system. Preliminary results from our group had already detected modifications in the cellular distribution of lactose-containing glycoconjugates when migrating and gonadal PGCs were compared (Fargeix *et al.*, 1980). In addition, the EMA-1 epitope shared by mouse, chick and quail PGCs is expressed in the chick according to a sexually dimorphic temporal pattern (Urven *et al.*, 1988). In this article we present comparative observations concerning the expression and distribution of a variety of mono-, di- and oligosaccharides at the critical stages of migration, proliferation and sexual differentiation of the chick germ cell line. Germ cell labelings were performed with a panel of plant lectins and with the anti NC-1/HNK-1 Mab, a marker of neural crest cells in birds and mammals (Tucker *et al.*, 1988), of quail germ cells (Tucker and Thiery, unpublished), and more generally of cells involved in tissue remodeling during embryogenesis (Tucker and Thiery, 1984).

Results

Among the panel of markers assayed in this study, those listed in Table 1 (*) do not bind germ cells at any stage of their embryonic development. Those reagents are lectins belonging to different categories in terms of sugar specificity, such as LCA (mannose-glucose), LPA (neuraminic acid), 2 lectins in the fucose group (UEA and LAF), 3 in the N-acetyl-galactosamine group (BSI-A4, SBA, SJA) and 2 recognizing mono- and trisaccharides in the N-acetyl-D-glucosamine series (BSII, STA).

The positive markers have been classified into 3 series according to their binding characteristics to PGCs, gonocytes, and/or sexualized gonia (Table 2).

Series 1 includes lectins binding early PGCs only, either when located in the germinal crescent (BSI-B4) or when they migrate through the dorsal mesentery (HPA, PHA, LAL). The fluorescence observed in PGC cytoplasm was very intense with BSI-B4 (Fig. 1A), contrasting with the faint staining mediated by HPA, PHA and LAL in migrating PGCs (not illustrated). From these observations it can be concluded that germ cells display sugar residues in the galactose series accessible to some lectins at precise premigratory and migratory stages. In addition, weak labeling by the PHA mixture of erythro-(E) and leuco- (L) agglutinins may indicate a transient occurence of sialylated complex oligosaccharides.

In *series 2*, Con A, LOA and PSA have the same binding properties to D-mannose and D-glucose residues. However, in every case the lectin-induced fluorescence was always more intense with Con A than with PSA and was weak with LOA. All markers bind early PGCs as previously described (Fargeix *et al.*, 1980) but also gonadal gonocytes (Fig. 1B), spermatogonia (Fig. 1C) and oogonia (Fig. 1D). Except in the testis where the interstitial tissue was not fluorescent, Con A, LOA and PSA also bound the epithelial and somatic cells of

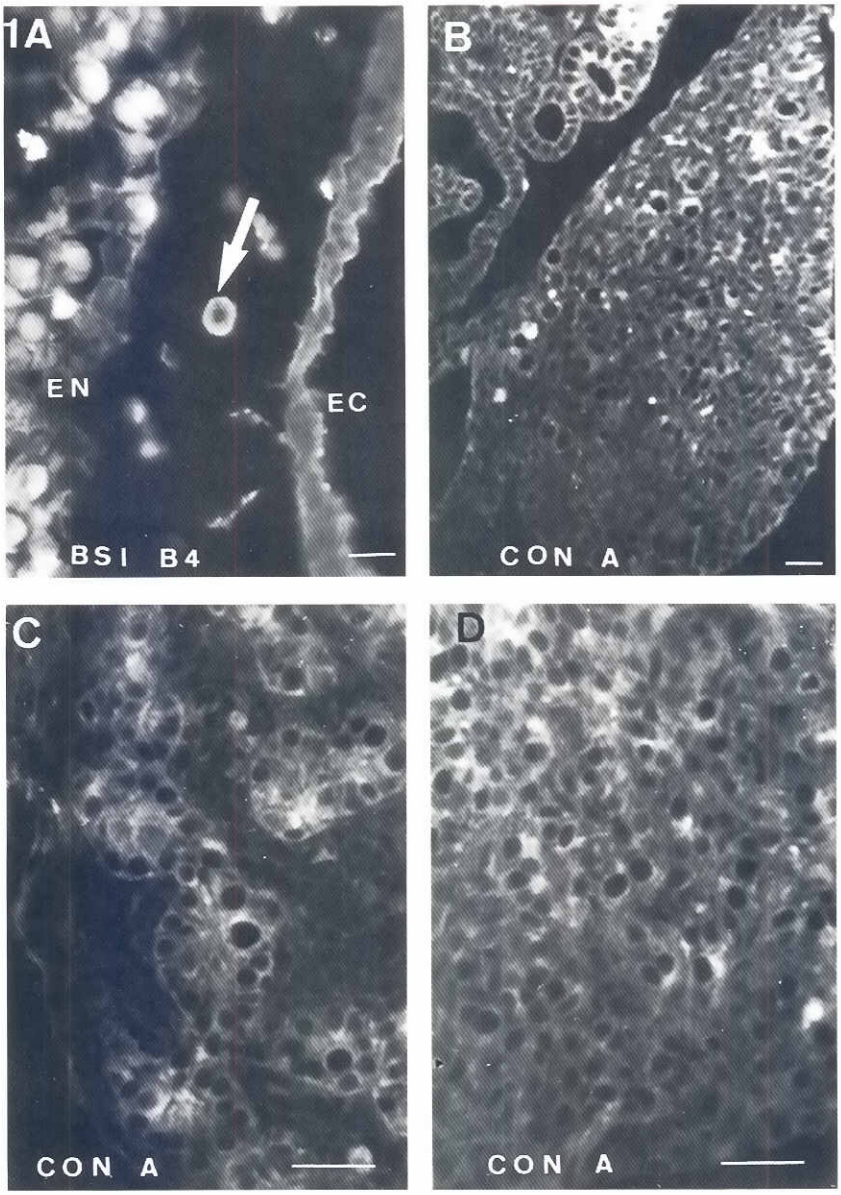

Fig. 1. Binding of BSI-B4 and Con A to PGCs and gonia (Paraffin sections). **(A)** *Cytoplasmic fluorescence of one PGC (arrow) in late germinal crescent (stage 9). The ectodermal cell membranes (EC) and endodermal cells (EN) are also fluorescent.* **(B, C, D)** *Gonocytes having reached the gonads bind Con A strongly in the undifferentiated gonad (B, stage 29) in the testis (C, stage 37) and in the ovary (D, stage 37). Note that the other cell types are highly fluorescent except in the testis. (Scale bars: 20μm).*

the undifferentiated gonad and of the ovary. Thus lectins from series 2 can be considered as non-specific, permanent markers of the germ cell line.

Series 3: a variety of lectins binding sugar residues in the galactose, lactose and glucosamine groups, respectively, as well as the NC-1 Mab were found to be stage-specific markers of the chick germ cell line. As previously observed with PoSA (Fargeix *et al.*, 1980), all these markers generate a strong cytoplasmic fluorescence in premigrating and migrating PGCs; when gonocytes settle in the developing gonad, the distribution and fluorescence intensity of binding sites are modified so that, in some cases germ cells no longer bind the markers. In sexualized gonads, most of binding sites are re-expressed in gonia, either in male and female or, predominantly, in spermatogonia (Table 2).

The variability of the expression of *N,N'-diacetylchitobiose and/or N-acetyllactosamine* sites is illustrated Fig. 2 by DSA and LEA. The exact specificity of LTA still remains unknown since fluorescence and hemagglutination induced by LTA could not be significantly inhibited by any of the haptens listed in Table 1. Although this lectin must recognize a more complex unidentified oligosaccharide, LTA receptor sites were absolutely similar to DSA and LEA as regards distribution and appearance. Every one of these markers confers a bright fluorescence on germ cells at early stages and during migration. The lectin binding sites either appear as scattered cytoplasmic granules (Fig. 2A) or are distributed all over the PGC cytoplasm. One or two bright fluorescent spots situated close to the nucleus can be observed in many PGCs (Fig. 2A, B, C). These spots probably correspond to intense glycosylation sites in the Golgi area. In the developing undifferentiated gonad, the fluorescence pattern changes drastically and becomes reduced to the cell membrane (Fig. 2E, F); such a reduction occurs progressively since it is possible to observe a mixture of non-, weakly and highly fluorescent

TABLE 2

PATTERNING OF LECTIN AND MAB BINDING TO GERM CELLS

				Post migration		
Series	Marker	Germinal crescent Stages 2-9	Migration Stages 19-25	Undifferentiated gonad Stages 25-30	Ovary Stage 37	Testis Stage 37
	HPA	-	±	-	-	-
1	PHA	-	±	-	-	-
	LAL	-	±	-	-	-
	BSI-B4	+++	-	-	-	-
	LOA	+	+	+	+	+
2	Con A	+++	+++	+++	+++	+++
	PSA	ND	+	++	++	±
	DSA	++	+++	++	++	++
	LEA	++	+++	+	++	++
	LTA	+++	+++	+	+(+)	++
	WGA	+++	++	+	±	++
3	PNA	±	++	±	±	±
	RCA	++	++	±	±	++
	PoSA	+++	+++	+	+	++
	ECA	+++	+++	+	+	+++
	NC 1	+++	++	±	+	+++

+++ intense fluorescence; ++ moderate fluorescence; ± weak fluorescence; - no fluorescence. ND: not determined

cells in the same gonad (Fig. 2E). However, it should be noted that some germ cells keep on displaying the bright spot observed at early stages. The re-expression of the cytoplasmic lectin binding sites occurs with these markers in the male (Fig. 2G) and in the female as well (Fig. 2H). The density of receptors to DSA, LEA and LTA is significantly higher in PGCs than in other cell lines. However those receptors can also be observed in the perichordal sheet (Fig. 2C and D) in the Wolffian duct basement membrane (Fig. 2C), in some mesonephric tubes (Fig. 2D) and in the basement membranes of seminiferous tubes (Fig. 2 G).

A similar variable labeling has been observed with WGA (Fig. 3A-F) which binds *chitobiose and/or N-acetylneuraminic acid.* The majority of cells including PGCs are WGA-positive at early stages (Fig. 3A,B,C). In the germ cells the density of WGA receptors is high enough to confer a uniform labeling on the cytoplasm (Fig. 3A,B). With the WGA-peroxidase conjugate, the receptors have also been observed as small brown granules scattered in the cytoplasm and as a large perinuclear granule corresponding to the bright fluorescent spot already described (Fig. 3C). After migration, WGA-receptor sites are restricted to this spot and to the gonocyte cell membrane (Fig. 3E). In the developing gonad and the dorsal mesentery, somatic cells display also similar residues distributed over the epithelial cell membranes and also on some small perinuclear spots of the gonad medulla cells. A re-expression of WGA receptors has been observed in spermatogonia (Fig. 3F) but not in the ovary, in which every cell type displays a weak non-specific fluorescence.

With 3 lectins belonging to the *galactose-lactose* group, we confirm the results previously obtained with PoSA (Fargeix *et al.*, 1980) of a weakening of germ cell fluorescence following migration. With PNA, which recognizes the dissaccharide Gal ß1→3 GalNAc, the fluorescence is rather moderate even in migrating PGCs and weak or non-existent at other stages. Receptors for RCA 120 specific for terminal ß-D galactose residues and for PoSA and ECA, which recognize both lactose and lactosamine groups, are abundant at early stages (Fig. 4 A,B,C,D,E), are lacking at stage 30 (Fig. 4C) and are abundant in spermatogonia (Fig. 4F).

The NC-1 epitope, which is identical to HNK-1, is a trisaccharidic chain associating *sulfated glucuronic acid* and *acetyllactosamine* through a ß1→3 linkage (Muramatsu, 1988a). The immunoreactivity of an IgM anti- NC-1 Mab with the chick germ cell line has been studied and revealed also to be stage-specific. As early as stage 2 the very first identifiable PGCs start segregating between the epiblast and the hypoblast and are immunolabeled (Fig. 5A). Some isolated or clustered hypoblastic cells were also NC-1 positive, namely in the extending marginal zone; however it was impossible to assign a germinal identity to those cells (Fig. 5A-C). During their migration to the gonadal primordia, PGCs could be readily detectable with the NC-1 Mab in cryostat (Fig. 5D,E) as well as in paraffin (Fig. 5F) sections. It should be noted that the immunofluorescence distribution was progressively reduced to the cell periphery when PGCs had reached the developing gonads (Fig. 5D-F) and that the somatic cells of the germinal epithelium also displayed a weak NC-1 positivity. The sexually undifferentiated gonad did not show any immunoreactivity with the NC-1 Mab contrasting with the neural crest cells contributing aortic plexus and enteric ganglia, and with some glomerular cells of the mesonephros (Fig. 6A). With the onset of sexual differentiation, spermatogonia recovered a high NC-1 immunoreactivity and the NC-1 epitope was present in the whole cytoplasm, making spermatogonia readily identifiable inside the seminiferous tubes (Fig. 6B,C). It should be noted that NC-1 Mab also reacted with the cells from the interstitial gland (Fig. 6C). By contrast, some sparse, rather cortical oogonia bound faintly the NC-1 Mab (Fig. 6D).

Discussion

Molecular probes such as lectins and monoclonal antibodies can be used to define precise structures by detecting various isomeric forms and linkage types in carbohydrate chains. Some lectins have also been characterized as exo- or endolectins according to their binding properties to end-chain sugar residues or longer internal sequences (Gallagher, 1984). In this respect, Mabs raised to carbohydrates behave as endolectins. As mentioned by Muramatsu (1988a, b), both marker types appear as precious tools in histochemical approaches to embryonal materials present in very low amounts, since they make it possible to detect carbohydrates even at the level of a single cell. Thus they are particularly adequate in an analysis of the germ cell line.

The results presented here document the expression and the cell distribution of a variety of carbohydrate residues and sequences in chick PGCs and sexualized gonia.

Some terminal monosaccharides such as L-fucose, N-acetylgalactosamine and N-acetylglucosamine could not be detected in this study at any stage of the germ cell embryonal life, indicating that these residues were lacking or inaccessible to our markers, the lectins from *Ulex europaeus I, Laccaria amethystina I, Bandeiraea (=Griffonia) simplicifolia* I-A4 and II, *Glycine max* and *Sophora japonica* respectively. Our observations and the fact that the NB10. 3B4 Mab

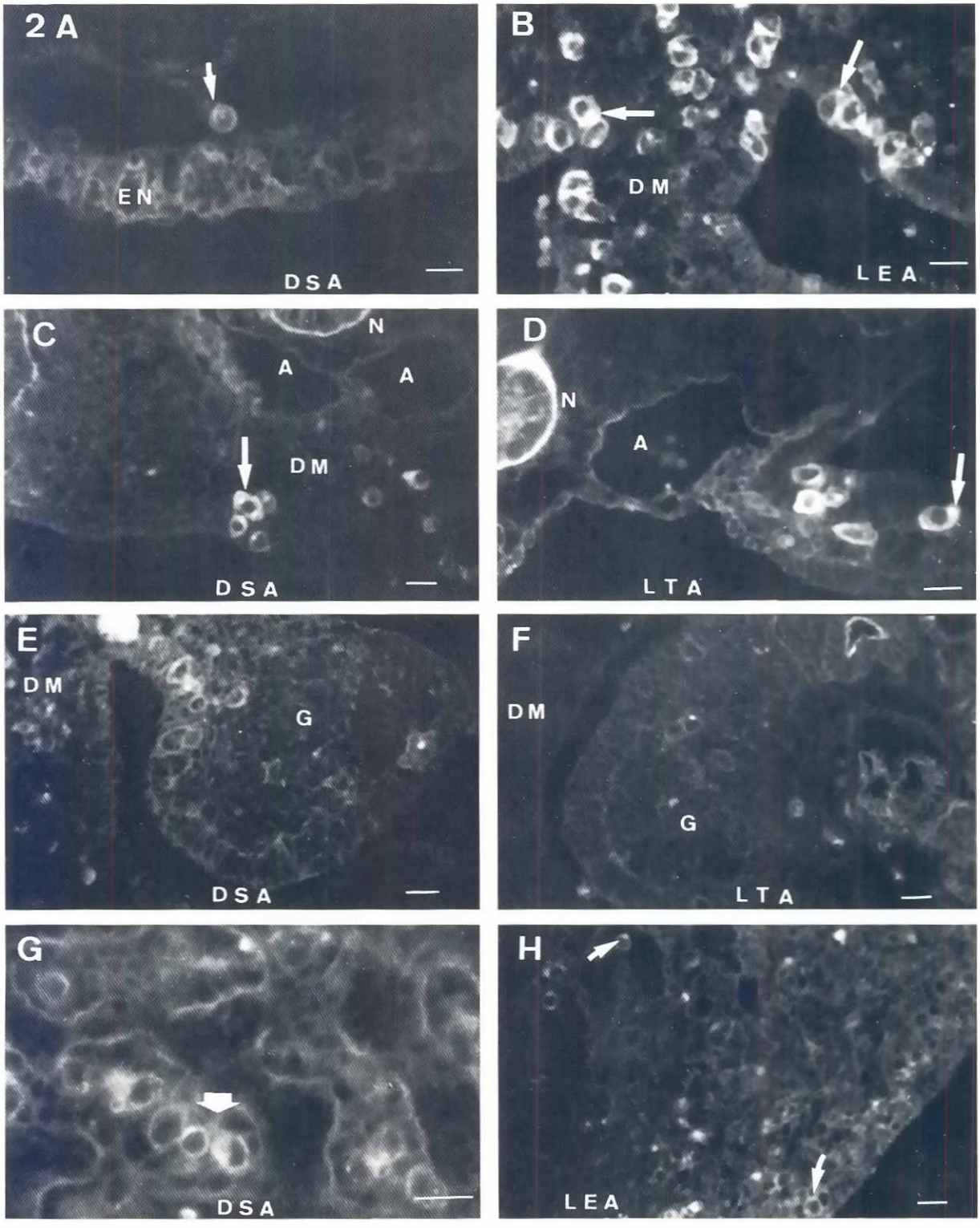

Fig. 2. Binding of DSA, LEA and LTA to PGCs and gonia. (A-F and H: Paraffin sections; G: cryostat section). **(A)** *DSA binds PGCs (arrow) and extra embryonic endoderm (EN) of the late germinal crescent (stage 10).* **(B, C, D)** *PGCs migrating across the dorsal mesentery display high fluorescence when stained by LEA (stage 22), DSA (stage 19) and LTA (stage 18), respectively. One or two very fluorescent spots can be seen in the cytoplasm of several PGCs (arrow). (N: notochord; A: aorta; DM: dorsal mesentery).* **(E,F)** *Decreasing of gonocyte fluorescence at post-migratory stages as seen with DSA (stage 27) and LTA (stage 28). Note that some germ cells are no longer fluorescent whereas others display a weak or high fluorescence. (G: gonad).* **(G,H)** *Most spermatogonia (large arrow) and cortical and medullar oogonia (small arrow) recover binding with DSA and LEA at stage 37. (Scale bars: 20μm).*

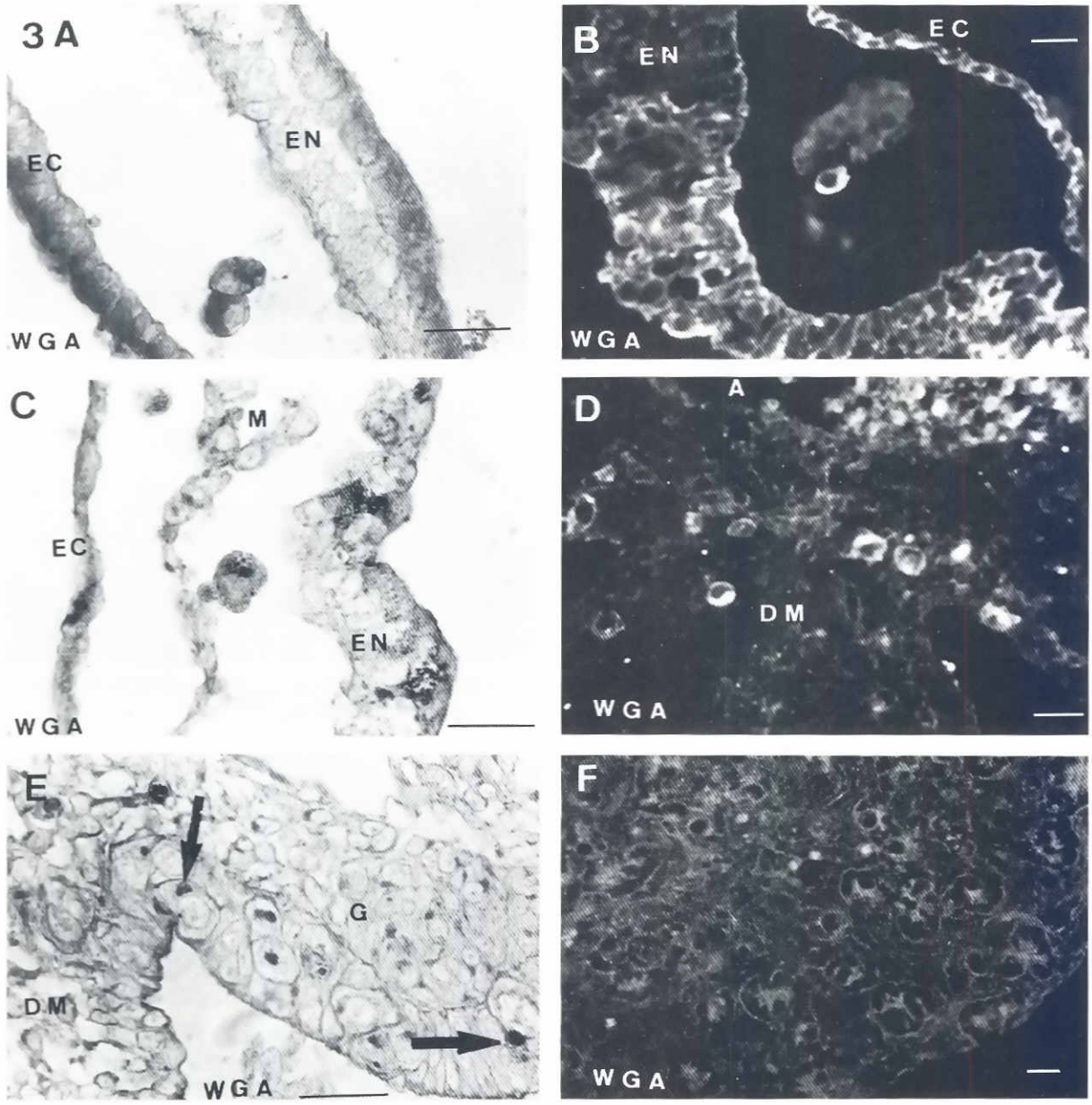

Fig. 3. Binding of peroxidase-WGA and of fluorescent WGA to germ cells (Paraffin sections). **(A, B, C)** *WGA binding to the cytoplasm of premigrative PGCs (stages 6,8 and 10 respectively). The distribution of the lectin binding sites is uniform in A and B and granular in C. Note that the perinuclear spot is apparent in B and C. (EC: ectoderm; EN: endoderm; M: mesoderm).* **(D)** *Migrating PGCs display an intense cytoplasmic fluorescence (stage 19). (A: aorta; DM: dorsal mesentery).* **(E)** *When PGCs have settled in the forming gonads (G) (stage 24), WGA-binding sites are restricted to the cell membrane and the perinuclear spot (arrow).* **(F)** *Re-expression of WGA-binding sites in the cytoplasm of spermatogonia (Testis, stage 37). (Scale bars: 20μm).*

(which recognizes a blood B group antigen in the Galα1-3Galß con-
[Fucα]
figuration) does not bind chick germ cells (Loveless *et al.*, 1990) indicate that avian PGCs do not display end-chain and/or lateral fucosyl residues. In contrast, fucose-containing sequences [Gal ß1-4 (Fuc α1-3) GlcNAc] have been identified in chick and mouse germ cells, as an EMA-1 antigen (Hahnel and Eddy, 1986; Urven *et al.*, 1988) considered as related to the SSEA-1/Le^x antigen (Fox *et al.*, 1981; Loveless *et al.*, 1990). Contrasting with rat and mouse germ cells (Fazel *et al.*, 1987; Kanai *et al.*, 1989) we did not observe any binding of several GalNAc specific lectins (BSI-A4, SBA and SJA) to chick germ cells. As in the mouse (Kanai *et al.*, 1989) end-chain GlcNAc residues could not be detected in the chick PGCs with BSII (=GSII, from *Griffonia simplicifolia*).

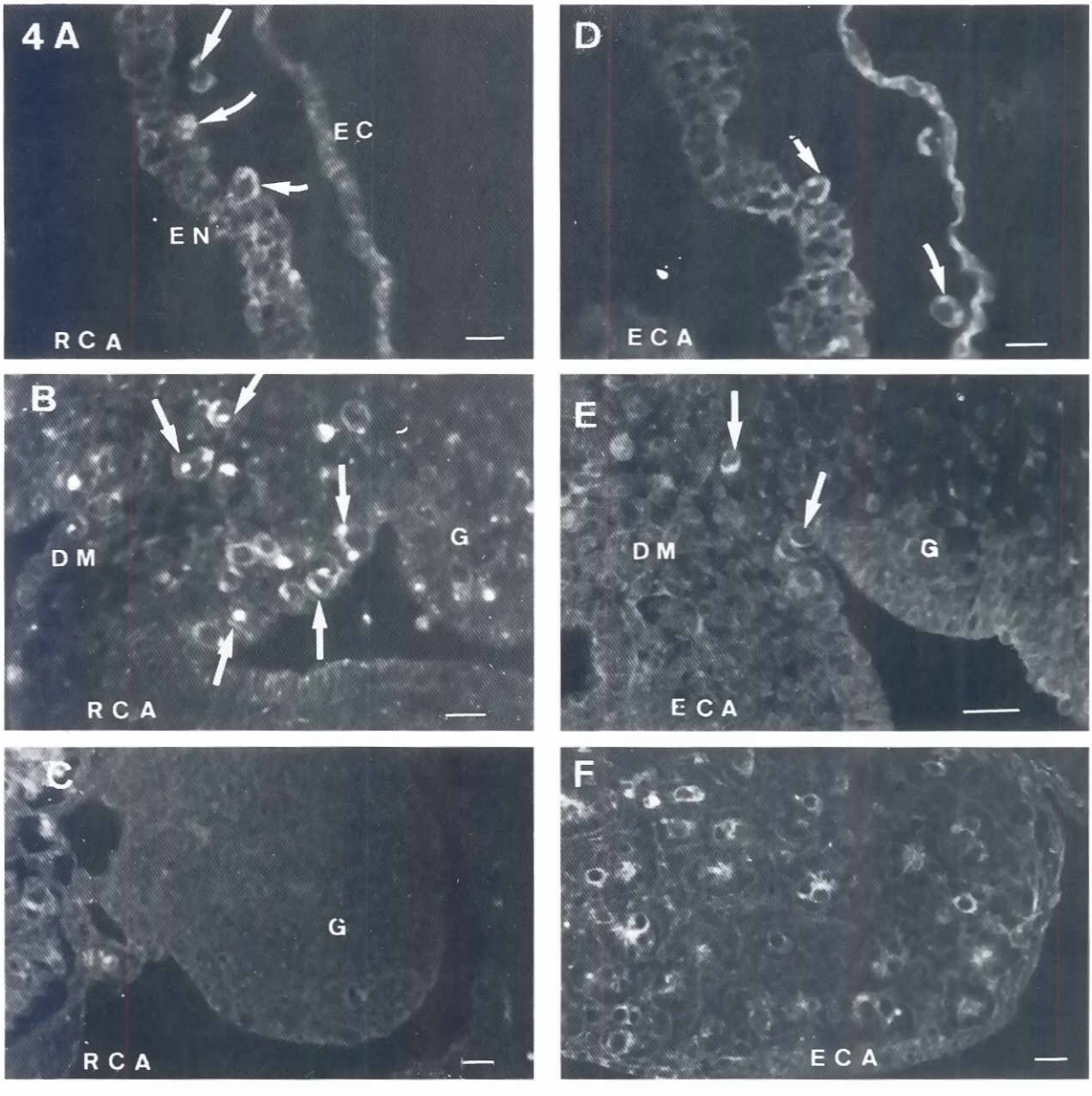

Fig. 4. Binding of RCA and ECA to PGCs and gonia (Paraffin sections). **(A)** *PGCs in the germinal crescent (stage 7); several fluorescent granules are seen in the cytoplasm (arrow). Note that RCA binds also to the extraembryonal ectoderm (EC) and endoderm (EN).* **(B)** *Migrating PGCs (stage 22) display a faint cytoplasmic fluorescence and one or two strongly fluorescent spots (arrow). The membranes of the epithelial cells lining the forming gonad (G) and the dorsal mesentery (DM) are also fluorescent.* **(C)** *In the undifferentiated gonad (G) (stage 30), the somatic and most germ cells do not bind RCA.* **(D)** *ECA binding sites are seen in the cytoplasm and the perinuclear spot (arrow) from several PGCs (germinal crescent, stage 7).* **(E)** *ECA faintly binds epithelial and mesenchymal cells and migrating PGCs (arrow) (stage 22).* **(F)** *Spermatogonia in a testis at stage 37 recover a bright cytoplasmic fluorescence (Scale bars: 20μm).*

Considered as facultative exolectins (Gallagher, 1984) Con A and other glucose/mannose-binding lectins such as PSA and LOA were found to label most of the avian embryonic tissues, including the germ cells at any of their developmental stages. These receptors are present in high amounts in the cytoplasm of early PGCs, gonocytes, oogonia and spermatogonia and must be considered as non-specific permanent markers of the germ cell line.

Carbohydrate sequences with α- and ß-galactose end-chains have been shown to be present in chick germ cells with markers such as BSI-B4, the ricin RCA 120 and PNA. The expression of α Gal residues seems to be very transitory in the chick since BSI-B4 binds only cytoplasmic receptors of premigrative PGCs. From their partial identity, the chick BSI-B4 receptor is probably identical to the mammalian GSI receptor (Gal α1→3 Gal) which is overexpressed in

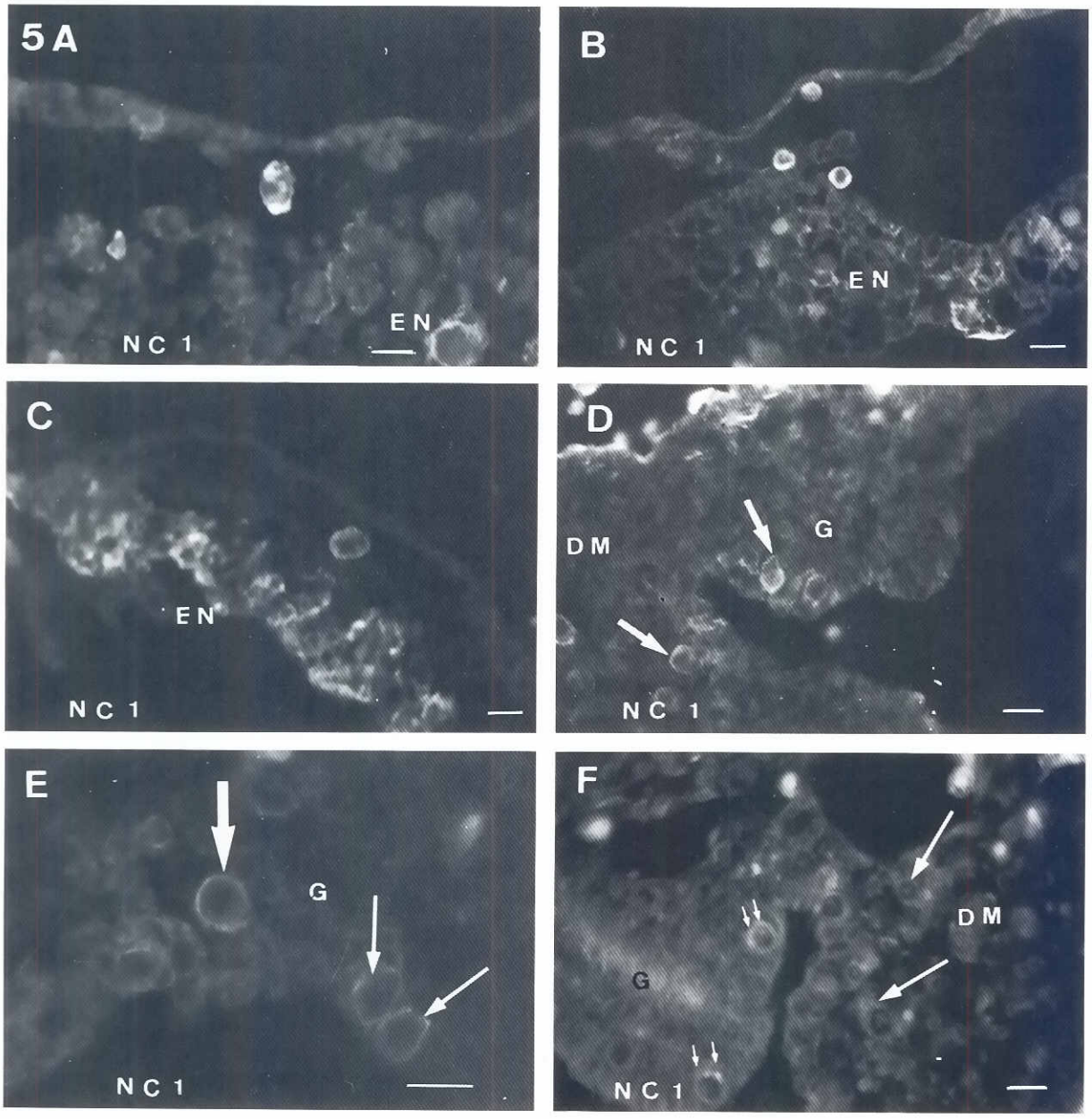

Fig. 5. Immunoreactivity of early and migrating PGCs with the NC-1 Mab (A,C,D,E: cryostat sections; B,F: paraffin section). **(A)** *As early as stage 2 (early primitive streak), morphologically identifiable PGCs, but also some extraembryonic endodermal cells (EN), are recognized by the NC-1 Mab.* **(B, C)** *Bright fluorescence of PGCs in the late germinal crescent (stage 9) and the early crescent (stage 4-5).* **(D, E)** *Migrating PGCs (arrow) are readily marked by the NC-1 Mab (stage 23), which also binds somatic cells to a lesser extent. Note that the fluorescence is mostly restricted to the plasma membrane.* **(F)** *Migrating PGCs (arrow) and gonocytes having settled in the gonad (double arrow) still remain NC-1 positive (stage 25). (G: gonad; DM: dorsal mesentery). (Scale bars: 20μm).*

human germ cell tumors (Muramatsu, 1988b). However the expression of other α Gal-containing antigens such as ECMA-2 previously detected in embryonic and adult mouse germ cells (Sato and Muramatsu, 1986) has not been investigated yet in the chick and cannot be absolutely ruled out. ß Gal have also been shown in the chick germ cells either as the terminal sequence Gal ß1→3 GalNAc recognized by the peanut agglutinin, or as a single monosaccharide bound by ricin. Those galactose residues were prominent at early stages prior to and during PGC migration.

With a panel of endolectins specific for lactose (Gal ß1→4 Glc), N-acetyllactosamine (Galß1→4 GlcNAc) and N-N' diacetylchitobiose (GlcNAcß1→4 GlcNAc), we have observed a stage-specific expression of these disaccharides in chick germ cells. From previous observations (Fargeix *et al.*, 1980) and the present work, it is seen

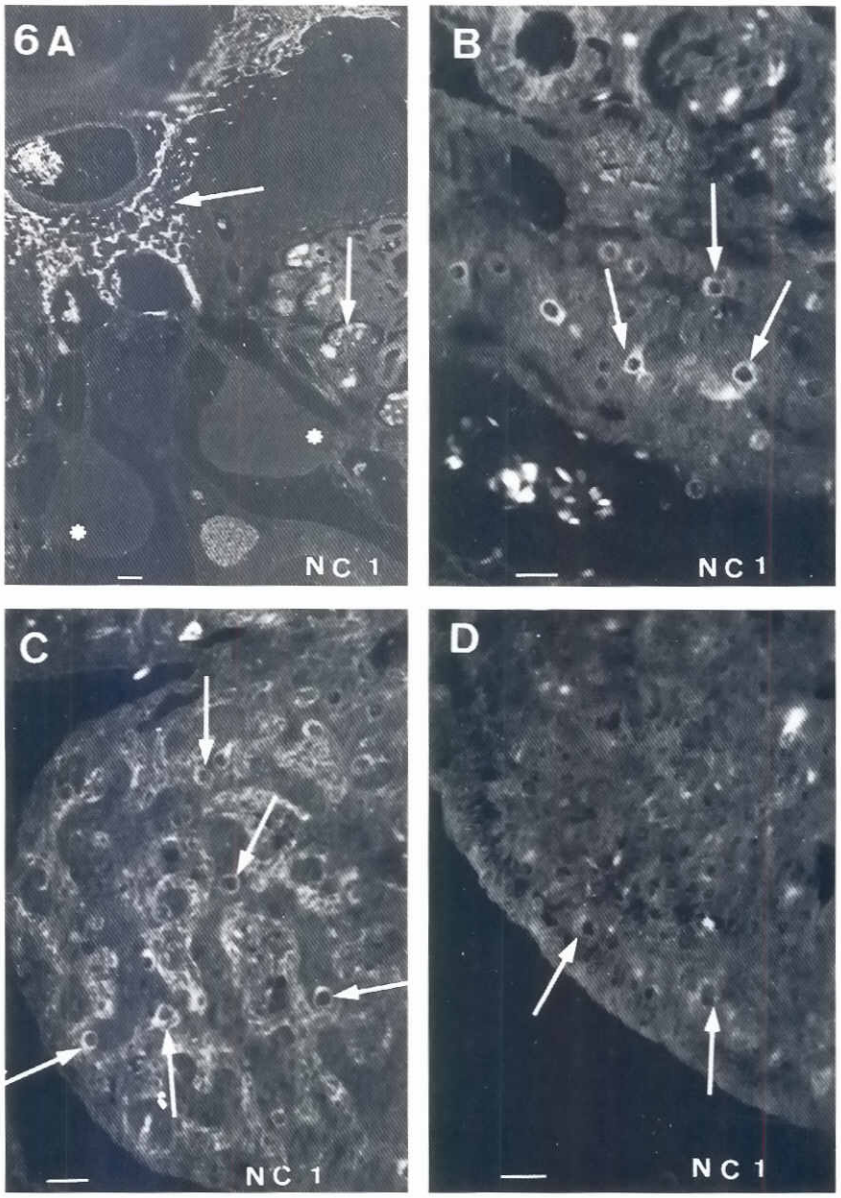

Fig. 6. Immunoreactivity of gonia to NC-1 Mab. (Paraffin sections). **(A)** *Contrasting with the neural crest cells and some mesonephros cells (arrows), gonads (*) do not contain any NC-1 positive cells at stage 29.* **(B)** *Testis, stage 35 (tangential section). Spermatogonia are now highly positive.* **(C)** *Testis, stage 35 (Cross-section). In addition to spermatogonia (arrows), NC-1 binds the interstitial tissue lying between the seminiferous tubes.* **(D)** *Ovary, stage 36. Few cortical oogonia (arrow) display a faint NC-1 immunoreactivity (Scale bars: A: 50 μm; B,C,D: 20 μm).*

that in the cytoplasm of premigrative and migrating PGCs, the density of these lectin receptor sites is high. In addition the lectins from *Datura stramonium, Lycopersicon esculentum, Lactarius torminosus* and WGA bound a perinuclear structure probably consisting of the Golgi complex (Didier, unpublished observations in transmission electron microscopy). A similar structure has also been observed in mouse PGCs and shown to be alkaline phosphatase positive (Ginsburg *et al.*, 1989b). When germ cells have settled in the developing gonads, the number of DSA, LEA, PoSA, WGA and ECA receptor sites decreased progressively so that gonads at stages 24-26 harbor a mixed population of labeled and unlabeled gonocytes. With the onset of sexual differentiation, a cytoplasmic reexpression of these carbohydrate chains occurs in spermatogonia and oogonia. Previous observations (Thorpe *et al.*, 1988; Loveless *et al.*, 1990) have led to the identification of linear and branched polylactosamine chains in early PGCs (stages 2-17). Our markers probably bind the Galß1→4GlcNAc sequence repeated in I (step) and i-Den and also present in the FC 10-2 antigens. In addition, we show that some such sequences are probably cut out or masked when gonocytes settle and start to proliferate in the developing gonads, and then are re-expressed in sexualized gonia at later stages.

Similarly we first show that the carbohydrate epitope recognized by the NC-1 Mab is expressed in the avian germ cell line in a stage-specific pattern. From immunocompetitive cytochemistry and immunoblotting, the NC-1 Mab raised to quail ciliary ganglion cells has been proved to be identical to the HNK-1 epitope displayed by a human leukemic cell line (Tucker *et al.*, 1984) and is assumed to recognize the sulfate 3 GlcAß1→3Galß1→4GlcNAc sequence borne by both glycolipids and glycoproteins (Muramatsu, 1988a). The NC-

1 antigen is shown here to be present at a high density at early stages of PGCs gathering in the germinal crescent and during migration through the dorsal mesentery. Contrasting with neural crest-derivatives such as aorta plexus and Remak ganglion, which still remain highly NC1-positive at stage 30, gonadal gonocytes no longer bind the NC-1Mab. The antigen will be re-expressed in sexualized gonia, especially in the spermatogonia cytoplasm. It should be noted that some somatic components of the germinal epithelium and the differentiating interstitial tissue were also found to react moderately with the anti NC-1 Mab.

Such a temporal variability in the expression of carbohydrate epitopes strengthens the idea of a remarkable control of glycosylation during avian and mammalian development (Muramatsu, 1988a, 1988b; Loveless *et al.*, 1990). In the chick germ cell line these alterations can obviously be related to differentiation events, metabolic changes and establishment of renewed relationships between the developing germ cells and their microenvironment. The last point likely involves modulated cooperations between adhesive glycoproteins or glycolipids (review in Thiery *et al.*, 1990) and their ligands through possible homophilic and heterophilic interactions (Loveless *et al.*, 1990). At this time our approach has not led to a precise identification of the intervening molecules. However a number of candidate adhesive molecules such as CAMs, SAMs, including integrins, cytotactin/tenascin and others which all display the HNK-1 epitope, have been proved to act in a coordinated fashion during embryogenesis and organogenesis (Thiery *et al.*, 1985, 1990; Canning and Stern, 1988; Künemund *et al.*, 1988; Riou *et al.*, 1988; Halfter *et al.*, 1989; Arsanto *et al.*, 1990; Levi *et al.*, 1990). However the spatial and temporal patterning of one or several of these molecules, and experimental *in vivo* and *in vitro* analyses of their functional interactions remain to be established in the chick germ cell system, keeping in mind that the same molecule may have contrary antiadhesive and cell-binding functions (Spring *et al.*, 1989).

The ligands for glycoproteins are either membrane or soluble carbohydrate binding proteins (*i.e.* endogenous lectins including addressins). They may also be involved in functional recognition systems (Loveless *et al.*, 1990). In chick germ cells, a ß-galactose lectin has been detected (S. Zalik, personal communication and work in progress in our group). Although embryonic lectins may have various functions and exert pleiotropic effects (Levi and Teichberg, 1990), it is conceivable that they could act as natural ligands for the prominent lactose/lactosamine-containing glycoproteins displayed, among other cell populations, by the germ cell line.

Materials and Methods

Preparation of embryos

Fertilized eggs were purchased from a local hatchery and incubated at 38.5°C. Embryos staged according to H.H. were fixed at stages 2, 5 (early germinal crescent), 9 (late germinal crescent), 22 to 30 (migratory and post-migratory stages). Gonads from male and female embryos were dissected out at day 10 (stage 37) and fixed separately.

Cytochemistry of lectin-binding sites

Embryos and gonads were fixed in Bouin's fluid and processed for paraffin embedding. Serial cross sections (6µm-thick) in the appropriate areas were selected on the presence of PGCs from morphological and cytological criteria. Sections were incubated with fluorescein isothyocyanate (FITC)-conjugated lectins diluted in 1% bovine serum albumin in phosphate buffer saline (PBS-BSA) for 1h in the dark, carefully rinsed in buffer and mounted in PBS-glycerol (10:90 v/v) prior to observations with the fluorescence microscope.

The fluorescent lectins used are listed Table 1. WGA, RCA 120 and ECA were purchased from Sigma. LPA, LEA, STA were from E.Y. Labs. The BS isolectins prepared according to Delmotte and Goldstein (1980) were a generous gift from F. Delmotte. All the other lectins were purified and fluorochrome-conjugated as previously described (Fargeix *et al.*, 1980; Guillot *et al.*, 1983; Gueugnot *et al.*, 1984). Controls of specificity were carried out by treating sections with a mixture of each fluorescent lectin and of 50mM of the appropriate hapten (Table 1).

The binding of peroxidase-conjugated-WGA (WGA-PO) prepared according to Gonatas and Avrameas (1973) has been investigated by treating paraffin sections with the conjugate diluted 1:80 (v/v) in PBS and revealing the enzyme activity by the classical diaminobenzidine-hydrogen peroxide reaction (Graham and Karnovsky, 1966).

Immunocytochemistry of the NC-1 epitope

Embryos or gonads were fixed in 3.5% formaldehyde, 0.05% glutharaldehyde in PBS (pH 7.4) for 1h at room temperature, incubated in graded sucrose solutions and embedded in Tissue Tek O.C.T. compound (Miles) as described by Crossin *et al.* (1986) with minor modifications. Specimens were frozen in liquid nitrogen. 6µm sections made with an Ames II cryostat were deposited on gelatin-coated slides and stored at -20°C until use.

Indirect immunofluorescence was performed by treating sections with the anti NC-1 Mab from ascitic fluid (Vincent and Thiery, 1984) diluted 1:80 (v/v) in 2% PBS-BSA, then by FITC-conjugated rabbit anti-mouse IgM (Nordic Imm) diluted 1:200. Control sections were treated by pre-immune mouse serum or by an irrelevant B2H3 anti brain hybridoma supernatant (a kind gift from R. Meiniel). Observations were made with a Leitz Orthoplan microscope equipped with the Ploemopak II epifluorescence system.

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