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Modulation of dye-coupling and proliferation in cultured rat thymic epithelium by factors involved in thymulin secretion

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ABSTRACT

Cultures of rat thymic epithelium were used to measure the effect of thymulin secretagogues on dye-coupling and proliferation. Dye-coupling was assessed after the injection of lucifer yellow dextran which cannot permeate the connexin pore of gap junctions and the smaller, permeant cascade blue. In addition to gap junctional communication, larger intercellular bridges were demonstrated by the transfer of lucifer yellow dextran between cells. The extent of intercellular communication was found to be influenced by both cell density and the number of passages. In control cultures, intercellular communication was reduced in cell groups of low (< 20 cells/group) or high cell densities (> 100 cells/group) compared with groups of 20–60 cells. The highest coupling indices were found in subcultures 20–30. Taking these factors into account, significant decreases in coupling index were observed after pretreatment of test cultures with factors known to influence the secretion of thymulin (5 U/ml interleukin 1 (α and β), 1 μM progesterone, 1 μM oestrogen, 1 μM testosterone, 1 ng/ml adrenocorticotropic hormone, 100 nM rat growth hormone) but 7.5 ng/ml thymulin had no effect on dye-coupling. The nonspecific gap junction uncoupler, octanol, abolished dye-coupling. Cellular proliferation, as measured by the uptake of tritiated thymidine, showed that the same factors that reduced coupling also increased proliferation. None of these factors affected the number of multinucleate cells present, except interleukin-1β which caused a significant reduction in the average number of nuclei per cell. Thus rat thymic epithelium in vitro provides a model for the study of the direct action of factors on cells of the thymic microenvironment.

Key words: Gap junctions; intercellular bridges.

INTRODUCTION

Human thymic epithelial cells can be classified morphologically into at least 6 types (Wijngaert et al. 1984), and similar cells have been found in all species so far studied (Kendall, 1991). Each group of thymic epithelia has a distinct range of functions in regulating thymocyte proliferation and maturation and the formation of the T cell repertoire. The initial steps of thymocyte differentiation are triggered by an increase in blast cell proliferation in the subcapsular cortex. This region also contains a high proportion of secretory epithelia whose factors influence the thymic microenvironment and some of which also enter the circulation to act at remote sites. Amongst these is thymulin, a well characterised nonapeptide whose production is highly restricted to the thymus (Savino & Dardenne, 1984, 1994; Kendall & Stebbings, 1994).

Epithelia communicate through gap junctions creating functional syncytia allowing the exchange of small molecules that participate in the coordination of cell function, and the transmission of electrical events. Gap junctionally coupled syncytia differ from the syncytia of the placenta in the limitations placed on intercellular movement by the size of the communicating pores. Functional communication has been demonstrated for the thymus in situ and for cultured thymic epithelium (Kendall, 1986; Alves et al. 1995). However, whether these communicating channels represent gap junctions or intercellular bridges (such as Witkin et al. 1995 described for the pituitary) is not clear. In this study, we have therefore used dyes of

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different molecular size to address these 2 possibilities, but used the term syncytia to encompass all cases of dye transfer between thymic epithelial cells.

The extent and transmission charactristics of syncytia can be rapidly altered. Intercellular permeability can be regulated by the rapid assembly and disassembly of gap junctions, or by modulation of the gap junctional pore permeability. The ensemble of intracellular signals, such as altered internal pH, temperature, internal calcium, NO, c-AMP, c-GMP and the activity of tyrosine and serine kinases, combine to effect changes (Cook & Becker, 1995). In addition, the available connexin pool can be regulated at the transcription level (Loewenstein, 1981; Kumar & Gilula, 1992). A further means of intercellular communication, intercellular bridges, are common in invertebrates and fungi (Robinson & Cooley, 1996). Similar junctions have recently been identified in mammalian gonadotropin-releasing hormone neurons (Witkin et al. 1995). Coupling of cells has the potential to integrate multiple independent cellular sensitivities into the functional syncytium. Thus syncytia can suppress a signal at source, recruit otherwise nonresponsive cells to participate in secretion, or amplify the response of a single cell.

In endocrine epithelia the major gap junction protein is connexin 43 (Meda et al. 1993) and its expression generally inhibits proliferation (Trosko et al. 1993). The converse is true in exocrine epithelia where connexin 32 is associated with the differentiated phenotype and proliferation is accompanied by increased expression of connexin 43 (Zhang & Thorgeirsson, 1994). An intermediate state occurs in haematopoietic tissues. Although connexin 32 is not found, connexin 43 and cell coupling are upregulated before lymphoid stem cells proliferate (Rosendaal et al. 1994; Krenacs & Rosendaal, 1994).

The role of cell coupling in the control of secretion also displays contrasting actions in different epithelia (Meda et al. 1993). In exocrine epithelia, increases in secretion are associated with decreased cell coupling whilst in endocrine epithelia, increased secretion is associated with raised intercellular coupling. Thymic epithelia display immunoreactivity to connexin 43, but not connexin 32 (Alves et al. 1994, 1995), suggesting that these secretory thymic epithelia have an endocrine phenotype. This is reinforced by the fact that blockade of intercellular coupling by octanol application reduces the level of secreted thymulin in vitro (Alves et al. 1996). In addition, intercellular communication may also occur in vivo since a functional syncytium was postulated from observations on the rapid movement of procion yellow across mouse thymus (Kendall, 1985, 1986).

Thymulin is almost exclusively secreted by thymic subcapsular/perivascular epithelial (type 1) cells, and by a subset of medullary, type 6, epithelial cells (Monier et al. 1980; Schmitt et al. 1980; Jambon et al. 1981; Savino et al. 1982; Gaudecker et al. 1997). Thymulin has been characterised biochemically (Bach et al. 1977) and shown to induce early differentiation markers on bone marrow derived thymocyte precursors, interacting specifically with T cytotoxic/suppressor cells (Kaiserlian & Dardenne, 1982) and enhances natural killer cell activity (Dokhelar et al. 1983; Bordigoni et al. 1984; Muzzioli et al. 1992). A single injection of physiological levels of thymulin into mice results in percentage decreases in $CD4^-/CD8^-$ and $CD8^+$ thymocytes within 2 h ($P < 0.001$), and a sustained decrease in $CD8^+$ thymocytes for 5 d (Kendall et al. 1992). Dardenne et al. (1978) found longer term effects for up to 10 d. Thymulin injections into rats and mice increase thymic weight and cellularity whilst restoring normal morphology in dwarf animals (Stebbings et al. 1993) and in ageing (Fabris et al. 1982). Many factors of the hypothalamic–pituitary–adrenal (HPA) axis (adrenocorticotrophin or ACTH, prolactin, growth hormone, sex steroids and corticosteroids) potentiate the release of thymulin (reviewed in Dardenne & Savino, 1990; Millington & Buckingham, 1992; Kendall & Stebbings, 1994). Cytokines such as interleukin (IL)-1 are also implicated with zinc in the control of thymulin secretion (Hadden et al. 1989; Coto et al. 1992).

Primary cultures of thymic fragments are heterogenous, but after only a few subcultures with appropriate media, they become $> 95\%$ epithelial and show a preferential expression of subcapsular/perivascular and medullary markers ($> 90\%$ of the cells). From this stage to the 30th subculture the cells are immunoreactive for the antithymulin antibody, 4β (Kurz et al. 1996). In this study, we have used these cultured thymic epithelial cells as an in vitro model in order to address the relationships between the secretion of thymulin, cell coupling and proliferation. Although 2 types of thymulin secreting epithelia are found within the thymus, this model may be considered appropriate for understanding regulatory mechanisms of thymic hormone release from the subcapsular/perivascular cortex since these type 1 cells both secrete thymulin and exist as a monolayer.

MATERIALS AND METHODS

Culture and incubation with stimulants

Thymic epithelial cell cultures were produced, characterised and passaged according to the method of Kurz et al. (1996). Four culture media (some with additions from Sigma) were used in the study: (1) DMEM/F12 alone; (2) a defined medium, (DMEM/F12 (Gibco), plus the following additions: 2 mM L-glutamine, 100 U/ml penicillin/streptomycin and 25 μg/ml transferrin); (3) the standard culture medium comprising the defined medium plus 10% horse serum, 5 μg/ml insulin, 10 ng/ml cholera toxin and epidermal growth factor (100 ng/ml); and (4) an RPMI-based medium with 2 mM L-glutamine, 100 U/ml penicillin/streptomycin, 0.25 mg/ml amphotericin and 1% fetal calf serum. Modulators were added to the defined medium from 1000-fold sterile stock solutions to create the following final concentrations: IL-1α and IL-1β, 5 U/ml; progesterone, oestrogen and testosterone, 1 μM; adrenocorticotrophic hormone $(ACTH)_{1-24}$, 1 ng/ml; rat growth hormone, 0.1 μM; octanol, 1 mM and thymulin, 7.5 ng/ml.

Cells to be used for immunocytochemistry were grown on sterile poly-d-lysine (Sigma) (1–10% v/v) coated glass coverslips. After 4 d, coverslips were fixed in acetone for 2 min at room temperature, air dried, wrapped in cling-film and stored at −30 °C for up to 1 wk, before processing.

Dye-coupling

Cultures of varying densities in 35 mm² dishes were equilibrated in DMEM/F12 alone for 2 h, and then incubated either in the defined medium or a test medium (created by the addition of the modulator to the defined medium) for 24–48 h. Cell cultures were transferred to microinjection apparatus (room temperature), and perfused with a modified Ringer's solution throughout the microinjection procedure which was limited to a maximum of 15 min. Before injection, dye was applied to the external surface of the cells. If any incorporation of dye within the cells was observed, then no injections were performed at that site. Dye coupling was assessed by the injection of lucifer yellow dextran (LYD) at a 2.5 mM concentration (Molecular Probes; mol. wt 10000 Da) and cascade blue (CB) at 5 mM concentration (Molecular Probes; mol wt, 644.77 Da) close to or into the nucleus of individual cells. The dyes were visualised (blue fluorescence for CB and yellow fluorescence for LYD) by simultaneous excitation at 365 nm (dichroic filter 395 nm, emission filter 420 nm) and the cells were observed by phase contrast microscopy.

To quantitate the extent of dye-coupling for each culture, the results are expressed as a coupling-index (CI) which is the average number of cells that are coupled to each injected cell, as demonstrated by the spread of fluorescent dye. Thus when there is no coupling the CI = 0.

Proliferation

Confluent cultures in 35 mm² dishes were equilibrated, and incubated as above for 48 h. A 1 μCi [^{3}H]thymidine aliquot (Amersham UK, code TRK 565) was added to each dish and incorporated for 5 h at 37 °C. The supernatant was discarded and the cells washed sequentially with phosphate buffered saline (PBS), water, 2 × methanol, 10% trichloracetic acid and water (5 min each). The cells were dissolved in 1 ml of 0.3 M sodium hydroxide (15 min), neutralised with 1 ml of 0.3 M hydrochloric acid and then 2 ml of sample was added to 10 ml scintillation fluid (Hydroluma, Baker Chemicals, code 8584) and the beta activity recorded. All experimental replicates (from passages 20–30) had paired control cultures from the same passage, and changes in proliferation were calculated relative to these.

The percentage of multinucleate cells was estimated under phase contrast illumination by counting the number of nuclei per single cell in a sample of > 200 cells. Multinucleate cells are distinct from syncytia by having closely apposed nuclei within a single cell body, whereas although syncytia also contain many nuclei, homogeneity of cytoplasm is restricted by gap junctions and intercellular bridges. The average number of nuclei per cell body was calculated from these data.

Immunocytochemistry

Wrapped coverslips were defrosted at room temperature for 10 min, air dried for 10 min before nonspecific binding was blocked with 0.1% casein and 0.1% bovine serum albumin (BSA). This blocking solution and all subsequent reagents were made up in PBS containing 1% Triton X (Sigma). Conventional immunocytochemistry was performed using optimally diluted monoclonal antibodies: 4β (raised to synthetic thymulin by Mary Ritter) and pancytokeratin (Sigma). For negative controls, the primary antibody was omitted. The reaction was visualised with

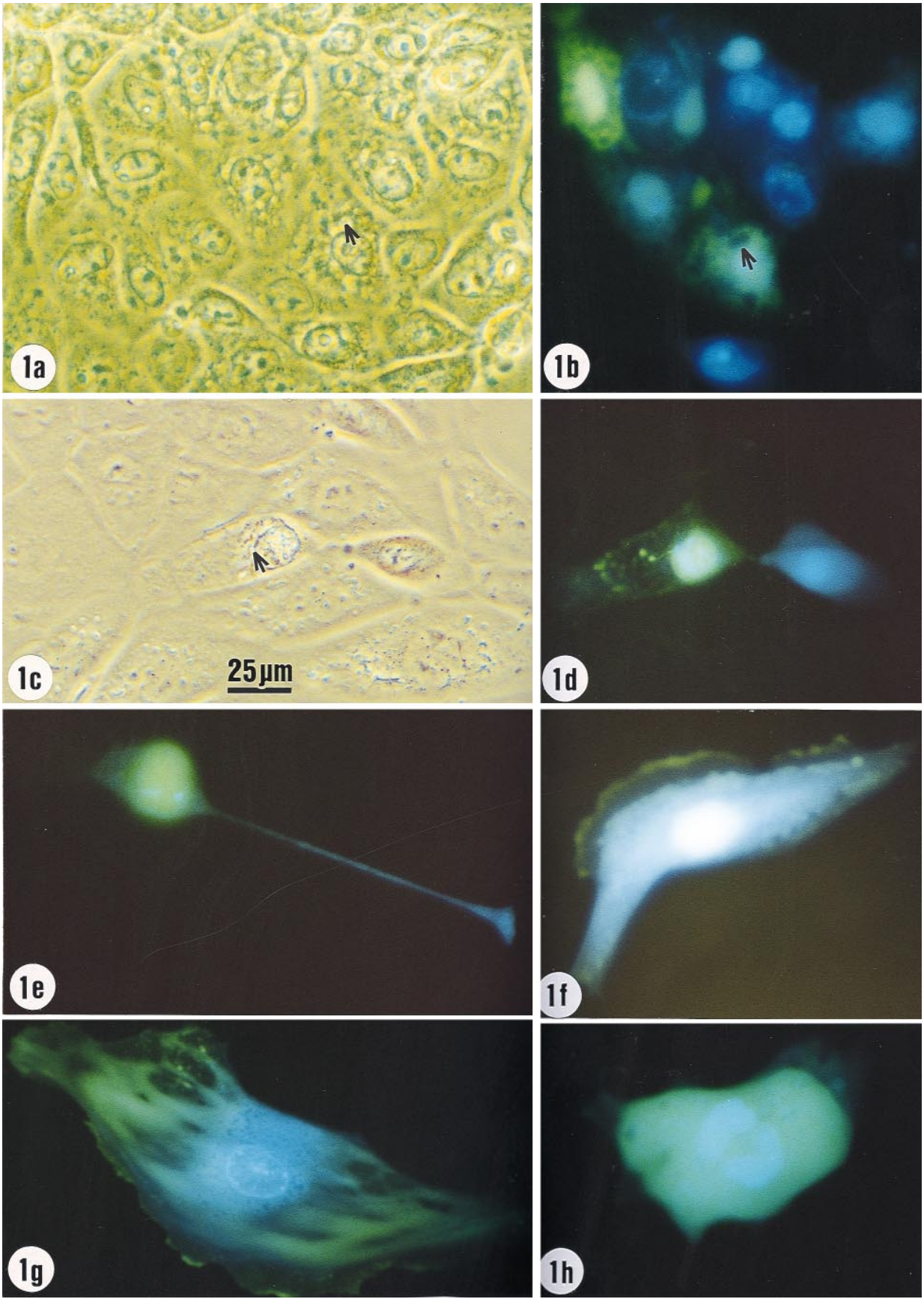

Fig. 1. Cell morphology and dye permeation (LYD, yellow and CB, blue) after injection (arrow indicates site) into cultured thymic epithelial cells. (*a*) Phase contrast and (*b*) fluorescent images of a large syncytium show intercellular transfer of LYD and CB. The connection between the LYD containing cells is not clearly visible probably due to its small diameter. (*c*) Phase contrast and (*d*) fluorescent images show 2 coupled cells where LYD is restricted to the injected cell. (*e*) Dye exclusion from a fine cytoplasmic process; (*f*, *g*) ruffled borders; (*h*) multinucleate cell. Bar = 25 µm.

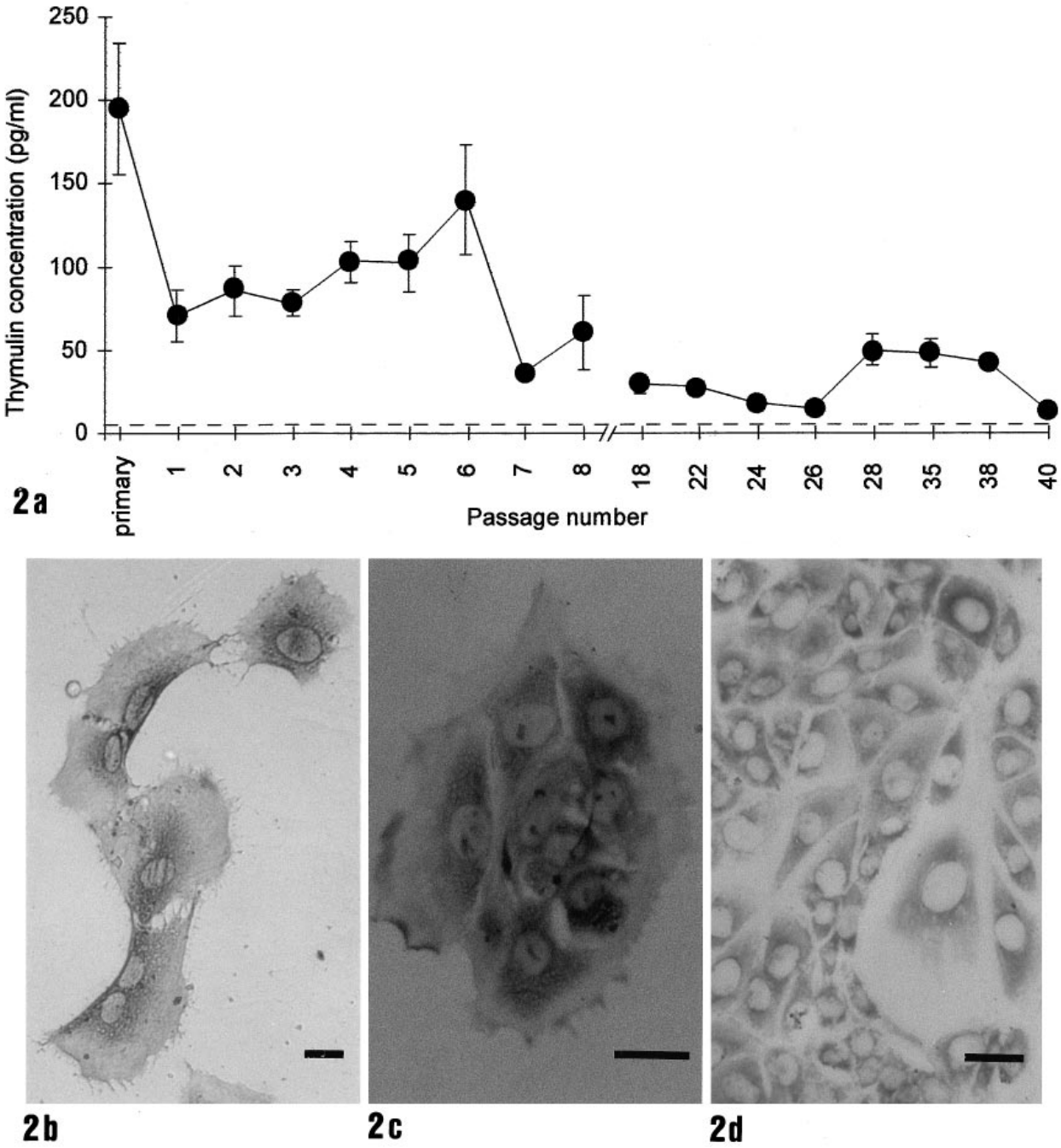

Fig. 2. (*a*) Thymulin concentration (mean ± S.E.M., n = 3–8) in the supernatant at different passage numbers. The ELISA lower detection limit is indicated by the broken line. (*b*, *c*, *d*) Immunoreactivity (black, nickel enhanced DAB) to the antithymulin antibody (4β) is illustrated in cells from passages 1, 27 and 36 respectively. Bars, 25 μm.

enhanced diaminobenzidine (Sigma) and viewed under bright field illmination.

Estimation of thymulin secretion

Thymulin levels in tissue culture supernatants from cells in passages 1–8 and 18–40 were estimated by ELISA. Each sample (120 μl) was incubated with 60 μl of polyclonal antibody (R4) raised to synthetic thymulin (Sigma) for 14 h at 4 °C, then transferred to a high binding plate where the remaining free antibody binds to a prepared coating. The coating was prepared by incubating 100 ng/well synthetic thymulin, in a high binding plate overnight at room temperature. The high binding plate was then washed with PBS plus 0.1 % Tween (Sigma) (PBS-Tween), blocked for 30 min at 37 °C with the same solution plus 1 % BSA and washed again before the addition of the pre-incubated samples. The reaction in the high binding

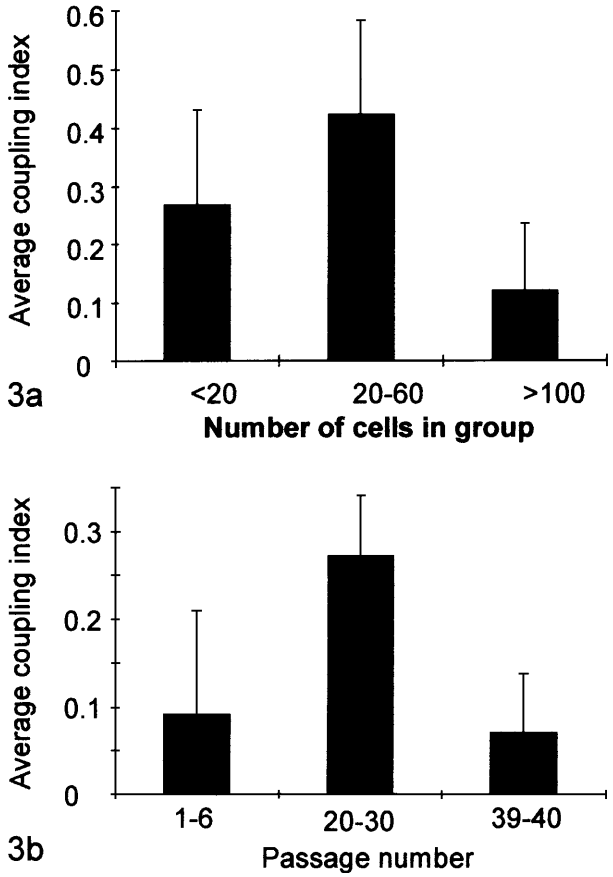

Fig. 3. Dependence of dye coupling (average CI + S.E.M., n = 28–107) on (*a*) monolayer clump size and (*b*) passage number.

plate continued for 14 h at 4 °C before washing with PBS-Tween. The excess antibody from the sample was reacted with a biotin-conjugated polyclonal goat antirabbit secondary antibody (Sigma, B7389). This was amplified with a streptavidin-biotinylated horseradish peroxidase complex (Amersham, RPN 1051) and developed by reaction with 3,3′,5,5′-tetramethylbenzidine (Sigma, T2885). The reaction was stopped with excess 2 M H_2SO_4 before visualisation in a spectrophotometer at 450 nm. Thymulin content was estimated by comparison with coreacted standard curves prepared by the addition of synthetic thymulin to tissue culture medium.

Statistical analysis

The results are expressed as mean ± standard error of the mean (S.E.M.), and significance analysed using Student's *t* tests.

RESULTS

Cellular morphology and dye permeation (*Fig. 1*)

Thymic epithelial cells (positive to anticytokeratin antibodies) in culture form a discrete monolayer of flattened cells (Fig. 1*a*), some of which become multinucleate. The intensity of fluorescence of the dyes was strongest at injection. Coupled cells could be immediately visualised although further dye movements did occur over the 10 min period of observation. It was expected that LYD would be retained within the injected cell, whilst CB permeated the syncytium. However, yellow fluorescence (LYD) was occasionally observed in some noninjected cells of a syncytium. This occurred in approximately 50% of syncytia with more than 2 cells (Fig. 1*a*, *b*), which in the defined media corresponded to approximately 10% of injections. During the course of this study, LYD transfer was not observed in syncytia with less than 3 members. Retention of LYD and transfer of CB in a manner typical of gap-junctional coupling is shown in Fig. 1*c*, *d*.

Injection of dyes sometimes revealed details of morphology not visible under phase contrast, such as changes in shape and apparent depth of cytoplasm, and compartmentalisation of cytoplasm. Fig. 1*e* shows a cell with a long cytoplasmic extension from which LYD was excluded even after 30 min. The proximal part of the extension is very faint due to its small diameter. Fig. 1*f*, *g* shows cells with prominent ruffled borders and Fig. 1*h* illustrates a multinucleate cell. The average diameter of mononuclear cells (49 ± 1 μm, n = 708) was significantly smaller than the average diameter of multinucleate cells (81 ± 4 μm, n = 75, $P < 0.00001$).

In control cultures, 15% of cells contained more than 1 nucleus. The average number of nuclei per cell was 1.22 ± 0.05. None of the modulatory panel significantly changed either the percentage of multinucleate cells or the average number of nuclei per cell except that IL-1β caused a significant reduction in the average number of nuclei per cell (1.17 ± 0.05, $P < 0.02$).

Thymulin secretion in control cultures

Thymulin levels in the supernatants fell during the first 7 passages, and maintained a steady level from passages 18–40 (Fig. 2*a*). Immunocytochemically, thymulin was detected in the cell body in the majority of cells in all passages from 1 to 43 (Fig. 2*b–d*). Positive reactions were also seen along cell borders and in cytoplasmic extensions (Fig. 2*c*).

Characterisation of dye-coupling in control cultures (*Fig. 3*)

When the local density of cells was < 20 cells/group, the CI was 0.27 ± 0.16 (n = 37). At 20–60 cells/group

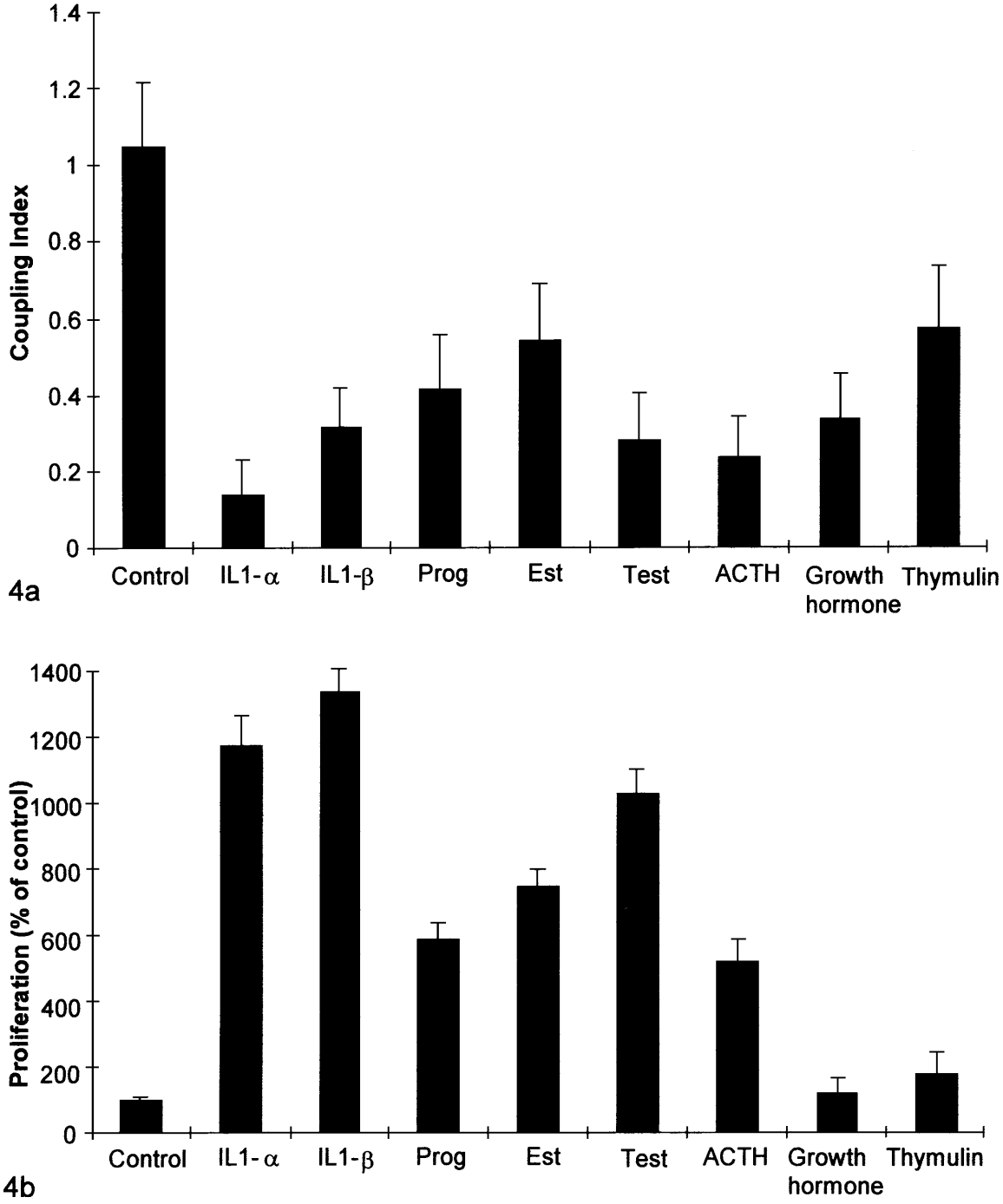

Fig. 4. (*a*) Change in CI (mean ± S.E.M., n = 30–106) after incubation with test panel. Prog, progesterone; Est, oestrogen; Test, testosterone. (*b*) Percentage change in proliferation relative to control, as in (*a*), where n = 5–7.

the CI was 0.42 ± 0.16 (n = 26). At higher cell densities (> 100 cells/group), the CI (0.12 ± 0.12, n = 25) was significantly lower ($P = 0.02$ to both lower densities). Therefore, for all further dye-coupling studies the results were taken from an equal number of injections made on cells at densities of < 100 and > 100 cells/group considered together.

Preliminary studies showed that dye-coupling in standard medium was very low in cultures which had undergone only a few passages (passage nos 1–6 averaged, CI = 0.09 ± 0.12, n = 28). The CI (0.27 ± 0.07, n = 107) was significantly higher ($P = 0.02$) in cultures after 20–30 passages, but in longer term cultures (39 passages), the CI (0.07 ± 0.07, n = 57) was significantly lower ($P = 0.001$ for subcultures 20–30 compared with subculture 39).

Studies using subcultures 20–30 showed that the culture medium influenced the CI. The removal of serum and growth factors from the medium (defined medium) significantly increased ($P = 0.0005$) the CI

(1.05 ± 0.17, n = 66) compared with standard medium (CI = 0.17 ± 0.06, n = 106), but the use of the RPMI-based medium gave similar values to the standard medium (CI = 0.18 ± 0.12, n = 39).

Modulation of coupling index by secretagogues of thymulin

The effects of the modulatory panel (as defined in the Materials and Methods) on the CIs are shown in Figure 4*a*. Interleukin α and β, progesterone, oestrogen, testosterone, ACTH and growth hormone decreased the CI whereas thymulin had no effect.

Cellular proliferation studies

The incorporation of [^{3}H]thymidine into cultures from passages 1–6 was only 16% of the incorporation into cultures from passages 20–30. The relative incorporations after incubation with the modulatory panel are shown in Figure 4*b*. Interleukin 1α (1180 ± 80%), IL-1β (1300 ± 60%), progesterone (610 ± 30%), oestrogen (760 ± 30%), testosterone (1060 ± 50%) and ACTH (520 ± 40%) all significantly increased ($P < 0.0001$) the incorporation of [^{3}H]-thymidine, whereas rat growth hormone and thymulin did not.

DISCUSSION

Our results contribute to the further characterisation of cultured rat thymic epithelium produced by the method of Kurz et al. (1996). The expression of thymulin is retained over many passages, albeit at a lower level, and remains fairly stable between passages 18–35 providing a good experimental model for thymulin-secreting cells. In vivo thymulin-secreting cells are the subcapsular/medullary types of epithelium (types 1 and 6, Wijngaert et al. 1984; Kendall & Stebbings, 1994). The morphological heterogeneity of cultured cells is suggestive of the presence of multiple phenotypes. For example, multinucleate cells are typically found in the cortex (Kendall, 1988), although in vivo they are not immunoreactive for thymulin (Gaudecker et al. 1997). Immunoreactivity for thymulin is not generally found in the differentiated cortex in vivo, thus a true differentiated cortical phenotype is not present. The pattern of CTES staining (Kurz et al. 1996), and the high passage numbers (> 40) achieved, also suggests that stem cells were present in the cultures.

In this study we demonstrate functional intercellular communication between rat thymic epithelial cells using fluorescent dye transfer. Most of these connections excluded the passage of a dextran-conjugated dye, but allowed the movement of CB, and are therefore consistent with gap junctions. Observations of the transfer of the dextran-conjugated dye between adjacent cells, however, must be mediated by larger conduits such as intercellular bridges (Robinson & Cooley, 1996). Cytoplasmic bridges have not been reported before for the thymus, and are unusual for somatic cells, although they have been demonstrated in neurons positive for gonadotrophin-releasing hormone in rat and monkey hypophyses (Witkin et al. 1995). These have a diameter of 0.1–0.5 μm while intercellular bridges in mammalian germline cells are 1–1.5 μm in diameter (Robinson & Cooley, 1996). The intercellular bridges observed here were absent after application of the nonspecific gap junction uncoupler, octanol, but also after treatment with IL-1α and IL-1β suggesting the possibility of endogenous regulation of these junctions. The mechanism of this blockade is not known although octanol can have nonspecific effects on intracellular calcium (Hossain & Evers, 1994) and pH (Pappas et al. 1996).

Thymic epithelial cultures containing syncytia connected by intercellular bridges may also have functional correlates in situ. Kendall (1986) postulated that the rapid appearance of multinucleate cells in the depopulated thymic cortex occurred when syncytia collapsed to multinucleate cells in the absence of thymocytes. This phenomenon of syncytial consolidation has been induced by application of cytochalasin D to interconnected spermatocytes in vitro (Russell et al. 1987, 1988) which then formed multinucleate cells.

The CIs determined here, for rat derived thymic epithelia, are considerably lower than the CIs for mouse and human thymic epithelia reported by Alves et al. (1995) as illustrated in Figure 5. They also observed species differences in the CIs. The lower CIs recorded in our study might be due either to species or procedural differences. High levels of connexin 43 immunoreactivity in sections of rat thymus were also shown by Alves et al. (1995). Corresponding studies on our cultured rat thymic epithelial cells have not been carried out to resolve whether the low levels of coupling in the rat thymic epithelial cell cultures is a result of low connexin availability, or is maintained despite a high intracellular connexin pool.

For cultures grown in identical media, changes in cell density and passage number caused positively correlated changes in CI and proliferation (Fig. 3). In most culture systems, there is a relationship between the rate of proliferation and the density of the cells.

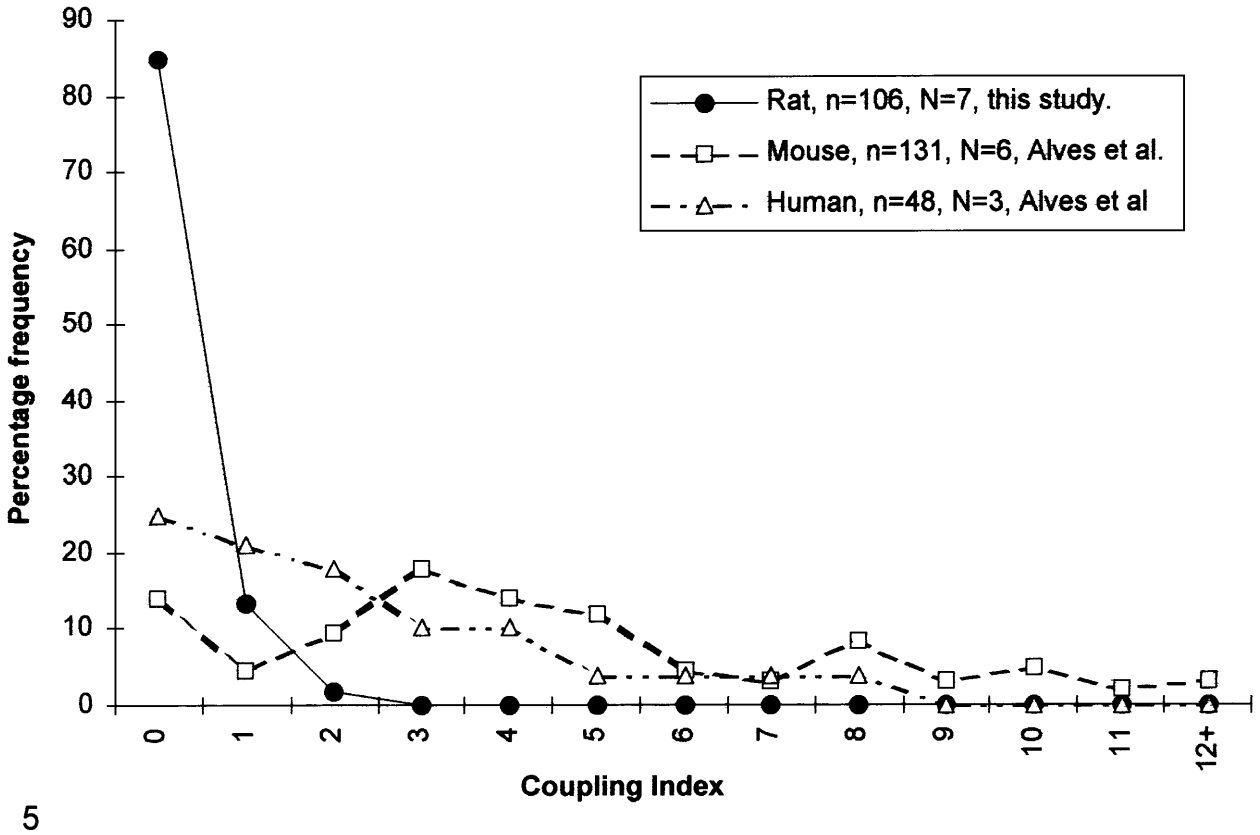

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Fig. 5. Comparison of data on frequency of CI in this study compared with data from Alves et al. (1995). n, number of cells; N, number of experimental replicates.

Both low and high cell densities usually cause low proliferation, the first by the absence of paracrine factors, and the second because of density-dependent inhibition. Intermediate density cells usually proliferate quickly in the log-phase of growth. The changes in CI at high densities were probably mediated either through contact-dependent mechanisms or by short lived diffusible messengers as the effects were restricted to the local group of cells and not dependent upon the average cell density across the dish. In contrast to this positive correlation, the effects of serum or secretagogues on CI and proliferation were negatively correlated, causing increased proliferation but decreasing the CI. Thus CI and proliferation may not be causally related in these cells. In addition it is difficult to assign a mode of action to serum induced effects which could be due to secretagogues or mitogenic components within the serum.

Thymulin is known to activate inhibitory feedback reducing its own release (Savino et al. 1983). This inhibitory feedback probably does not involve either modulation of proliferation or intercellular communication as these parameters were unaffected by the addition of thymulin. Thus the decreased CI and increased proliferation produced by secretagogues do not represent a negative feedback mechanism activated by thymulin release.

The enhancement of proliferation and suppression of dye coupling in response to secretagogues is a relationship typically found in exocrine epithelia. Cultured thymic epithelial cells also behave like endocrine epithelia as secretion is inhibited by gap-junction blockade with octanol (Alves et al. 1995). However, the study illustrating this included fetal calf serum in the medium which may complicate the responses. Other evidence suggests that thymic epithelia do not belong to the exocrine family as they express connexin 43, not connexin 32. This may mean that they have an intermediate phenotype. There are precedents for this in thyroid follicular cells which express both connexin 43 and 32, and have an atypical method of secretion (Meda et al. 1993), as well as the examples of the connexin complement of haematopoietic tissues mentioned in the introduction (Krenacs & Rosendaal, 1995; Rosendaal et al. 1996). Thymic epithelia do not secrete into ducts, and only a minority of cells have access to a blood vessel. Therefore, their secretion, mainly into the tissue parenchyma, does not conform to exocrine or endocrine definitions. This may explain why the pattern of changes in dye-coupling, proliferation and secretion does not fit

either of those characterising exocrine or endocrine cells.

It is well established that thymic epithelial cells bear receptors for hormones, cytokines and other factors that influence thymic function (reviewed in Kendall, 1991; Kendall & Clarke, 1994). This study has demonstrated that cultured thymic epithelial cells form a syncytium. Since receptors are functionally shared between communicating cells, those on individual cells could transduce signals through the multicellular system. In this way, otherwise unresponsive cells can be recruited (Combettes et al. 1996) or responses modified by amplification or inhibition (Munarisilem et al. 1995).

There is much evidence from work on electrically coupled neurons to suggest that changes in the size of the syncytia or its junctional resistance (Santos-Sachi, 1991; Cook & Becker, 1995) determine the way in which electrical signals are summated. Thus the integration of membrane voltage and/or exchange of intracellular components through the local syncytia we identified in thymic epithelium could influence the maturation and/or differentiation pathways of thymocyte development.

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