Epithelial interactions in *Hydra*: apoptosis in interspecies grafts is induced by detachment from the extracellular matrix

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Summary

Apoptosis plays an important role in immunity and is widely used to eliminate foreign or infected cells. *Cnidaria* are the most basal eumetazoans and have no specialised immune cells, but some colonial cnidarians possess a genetic system to discriminate between self and non-self. By grafting epithelia of different species we have previously shown that the freshwater polyp *Hydra* eliminates non-self cells by phagocytosis. Here we have investigated whether apoptosis is involved in the histocompatibility reactions. We studied epithelial interactions between *Hydra vulgaris* and *Hydra oligactis* and show that a large number of apoptotic cells accumulate in the contact region of interspecies grafts. Histological analysis of the graft site revealed that displacement of the endodermal layer of *Hydra vulgaris* by endoderm from *Hydra oligactis* coincided with impaired cell–cell and cell–matrix contacts. We therefore suggest that in interspecies grafts, apoptosis is induced by the detachment of epithelial cells from the extracellular matrix (anoikis) and not by a discriminative allorecognition system.

Key words: *Hydra,* histocompatibility, allorecognition, apoptosis, anoikis, extracellular matrix, septate junction.

Introduction

Apoptosis plays an important role during the development and function of the vertebrate immune system and in execution of its functions by eliminating non-self and infected cells (for a review, see Jacobson et al., 1997; Maier et al., 2001). The apoptotic machinery is conserved throughout the animal kingdom (for a review, see Meier et al., 2000) and can be traced back in evolution from vertebrates to the freshwater polyp *Hydra* (Cikala et al., 1999). The molecular effectors of apoptosis seem to be permanently expressed but inactive in all animal cells (Weil et al., 1996). Presence or absence of death-suppressing or death-promoting signals can trigger programmed cell death by activating the suicide machinery. External signals suppressing apoptotic signalling include soluble or plasma-membrane-bound factors. In addition, there is evidence that survival of epithelial cells requires appropriate contacts with the extracellular matrix (Meredith et al., 1993). Impaired cell-matrix contact leading to induction of programmed cell death has been termed 'anoikis' (Frisch and Francis, 1994) and was found to be involved in a wide diversity of tissue-homeostatic, developmental and oncogenic processes.

In the wild, some invertebrates undergo so called 'natural transplantations', when they experience close contact with tissue from other individuals of the same or related species during larval settlement and growth (Rinkevich, 1996). Due to the presence of mobile multipotent stem cells in these animals, tissue fusion may lead to formation of chimeric individuals. To prevent such somatic and germ cell parasitism, efficient detection and defence mechanisms against non-self cells are thought to have evolved (Buss, 1982). *Cnidaria*, as the most basal eumetazoans, have no specialised immune cells. However, as shown in the hydroid *Hydractinia echinata*, contact between two colonies leads to either fusion or rejection. Since *Hydractinia* fuse only with self and reject all non-related conspecific tissue (Frank et al., 2001), they seem to possess a genetic system that can discriminate between self and non-self (Mokady and Buss, 1996). The solitary growing freshwater polyp *Hydra* also was suggested to have such a system (Bosch and David, 1986) and grafts between different *Hydra* species have long been considered a model for the ancestral form of transplantation immunity (Kolenkine, 1958; Kanaev, 1969; Campbell and Bibb, 1970). In heterografts between *H. attenuata* (=*H. vulgaris*) and *H. oligactis* we observed an increased number of phagocytozing epithelial cells, located within the contact zone, that selectively eliminate cells from the other species (Bosch and David, 1986). This observation suggested that a fundamental aspect of immunity – the ability to distinguish self from non-self – is present in *Hydra*. This was surprising since promiscuous fusion between individual *Hydra* polyps never occurs naturally and, therefore, the risk of cell lineage parasitisms is very low in *Hydra*.

To resolve the issue of the presence of a discriminative allorecognition system in *Hydra*, we reinvestigated tissue interactions between *Hydra vulgaris* and *Hydra oligactis*. We

observed that a large number of apoptotic cells accumulate in the contact region of interspecies grafts. Initiation of apoptosis at the graft site is correlated with impaired cell–matrix and cell–cell contacts. We report elsewhere (S. G. Kuzetsov and T. C. G. Bosch, manuscript in preparation) that contact to allogeneic tissue does not evoke any response in *Hydra* in terms of phagocytosis and elimination on non-self cells. We therefore suggest that, contrary to the previous view, in interspecies grafts apoptosis is induced by impaired cell–cell or cell–matrix contacts and not by a discriminative recognition system.

Materials and methods

Animals

Experiments were carried out with *Hydra vulgaris* Pallas strain Basel and *Hydra oligactis* Pallas originally obtained from Dr Charles David, Munich. Animals were cultured according to standard procedures at 18°C. Experimental animals were starved for 24 h before use. *H. attenuata*, the species used in a previous study (Bosch and David, 1986) has meanwhile been identified correctly as *H. vulgaris* (Holstein et al., 1990).

Transplantation procedure

Grafts were produced as described (Fujisawa et al., 1990). Briefly, animals were cut transversely in the mid-gastric region, and complementary apical and basal halves of two polyps were threaded on a fishing line and held together by sleeves of polyethylene tubing to facilitate healing. After 1–2 h grafts were taken off the fishing line and maintained under standard conditions without feeding.

Preparation of mixed-cell aggregates

Polyps were mechanically dissociated into a cell suspension in dissociation medium and reaggregated as described (Gierer et al., 1972). To prepare heteroaggregates, about 250 polyps of *Hydra vulgaris* and *Hydra oligactis* were separately dissociated in 20 ml dissociation medium. Tissue pieces and large cell clusters were allowed to sink for 30 s. Polypropylene microcentrifuge tubes (Roth) were filled with 400 µl of the remaining cell suspension and centrifuged at 1200 g for 5 min at 18°C. The size of the resulting pellets allowed us to assess and equilibrate cell concentrations in both preparations. For heteroaggregates, equal volumes of *Hydra vulgaris* and *Hydra oligactis* cell suspensions were carefully mixed together, transferred to microcentrifuge tubes and pelleted as described above. The dissociation medium was then diluted with the hydra medium to 75%, 50%, 33%, 25%, 10%, 5% in 6, 8, 20, 24, 32 and 36 h after reaggregation, respectively. 44 h after reaggregation, cell aggregates were transferred into hydra medium. Homoaggregates were prepared in each experiment to control the size and the quality of resulting aggregates.

Tissue labelling with fluorescent beads

Fluorescent latex beads labelled with fluorescein isothiocyanate (FITC) were used to label hydra cells by phagocytosis as described previously (Technau and Holstein, 1992). Endodermal epithelial cells could be selectively labelled by injecting a 2.5% bead suspension into the gastric cavity using a glass capillary. For selective labelling of ectodermal epithelial cells, 24 h starved *H. vulgaris* polyps were incubated for 15 h in a 0.025% suspension of beads.

Transmission electron microscopy

Polyps were relaxed in 2% urethane prior to fixation in 3.5% glutaraldehyde in 0.05 mol l^{-1} cacodylate buffer, pH 7.4, for 18 h at 4°C. After washing with 0.075 mol l^{-1} cacodylate buffer for 30 min, animals were postfixed with 1% OsO$_4$ in 0.075 mol l^{-1} cacodylate buffer for 2 h at 4°C. After additional washing for 30 min the tissue was dehydrated in ethanol and embedded in Agar 100 resin (Agar Scientific, Ltd., Essex). Semithin sections were stained according to Richardson et al. (1960) with a solution containing 0.5% Methylene Blue, 0.5% borax, and 0.5% Azur II in ddH$_2$O at 60°C for 1–2 min. Ultrathin sections were contrasted with 2.5% uranylacetate and lead citrate solution (prepared freshly from lead acetate and sodium citrate) for 2 min (Reynolds, 1963) and analysed using a transmission electron microscope CM10 or EM 208 S (Philips).

Immunohistochemistry using species-specific antibodies

Species-specific mouse polyclonal antisera were raised against membrane fractions of *H. vulgaris* and *H. oligactis* (Samoilovich et al., 2001). To remove cross-species reacting antibodies, the antiserum was preincubated with fixed cells of the opposite species overnight at 4°C. For light microscopy, polyps were fixed with 4% formaldehyde in hydra medium, dehydrated in ethanol and embedded in histoplast-S (Serva) according to the manufacturer's protocol. 6 µm sections were prepared, dehydrated in ethanol and stained with the polyclonal mouse antisera (diluted 1:500) prior to detection with a FITC-labelled sheep-anti-mouse secondary antibody (Boehringer Mannheim Biochemica). Sections were then counterstained with Toluidine Blue. Whole-mount staining was performed as described for paraffin sections. For immunostaining on ultrathin sections, unspecific binding sites were blocked by incubating the grids in PBS containing 0.1% Triton and 3% BSA (PBS-Triton-BSA) for 30 min. Incubation with the primary antibody in PBS-Triton-BSA was done at 37°C for 1 h. Preparations were washed for 4× 5 min with PBS-Triton-BSA and incubated with the secondary goat-anti-mouse antibodies (Sigma) coupled with 10 nm large gold particles (1:20 dilution in PBS-Triton-BSA) for 40 min at 37°C. Preparations were then washed 4× 5 min in PBS-Triton-BSA followed by washing 4× 1 min with ddH$_2$O. The preparations were contrasted with uranylacetate and lead citrate as described.

Detection of programmed cell death

Hydra cells undergoing apoptosis were detected as described previously (Kuznetsov et al., 2001). Animals were stained in 0.1 µmol l^{-1} Acridine Orange, a fluorescent dye that is widely used to specifically highlight apoptotic cells in a
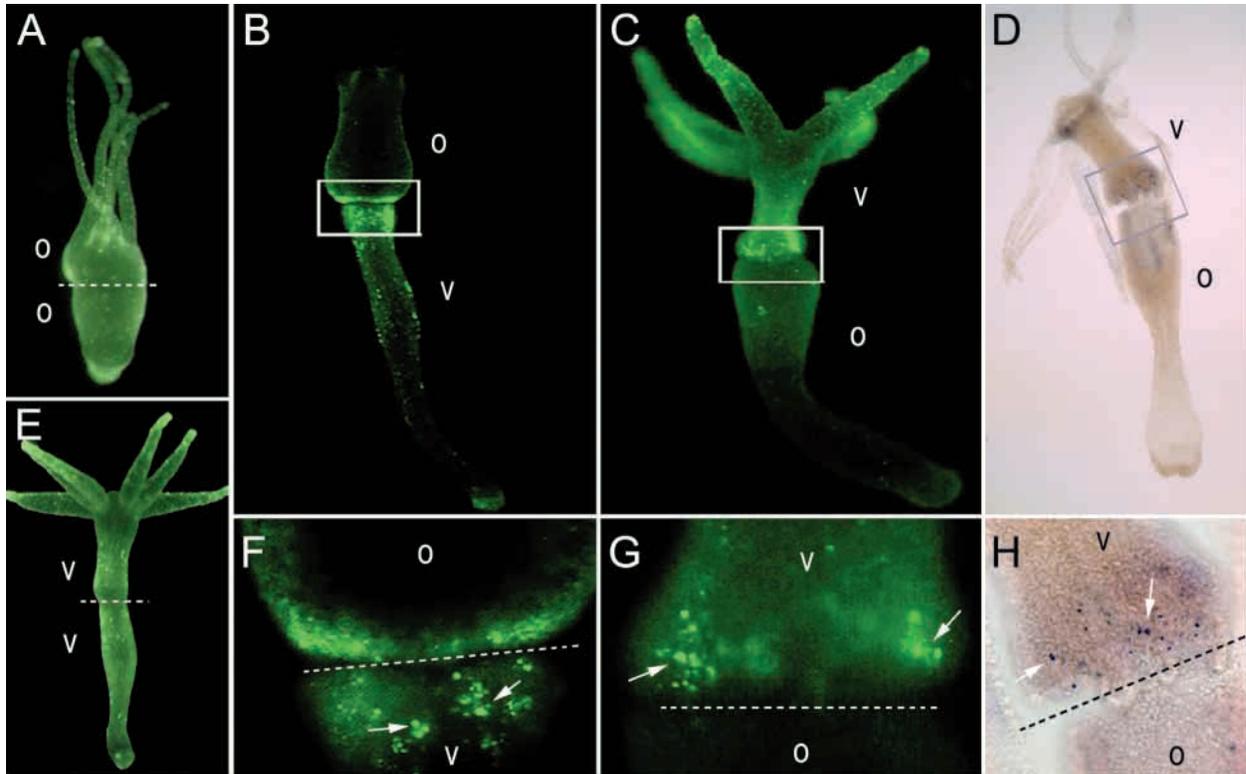
Fig. 1. Apoptosis is induced at the graft border in heterografts. Control (24 h old) homografts of *H. oligactis/H. oligactis* (A) and *H. vulgaris/H. vulgaris* (E), and heterografts *H. oligactis/H. vulgaris* (B) and *H. vulgaris/H. oligactis* (C) vitally stained with Acridine Orange. (D) *H. vulgaris/H. oligactis* heterograft stained by the TUNEL method. (F–H) Graft sites indicated by the frames in B–D, respectively, shown at higher magnification. O, *H. oligactis* tissue; V, *H. vulgaris* tissue. Broken lines indicate the graft border. Arrows show Acridine Orange- or TUNEL-positive cells undergoing apoptosis. The cells in focus are localized in the ectodermal epithelium. Note that most of them are apparently restricted to the *H. vulgaris* tissue adjacent to the graft border.

variety of organisms including *Hydra* (Cikala et al., 1999). DNA fragmentation, as an indicator of apoptosis, was detected by TUNEL [terminal deoxynucleotidyl transferase-mediated digoxigenin (DIG)-dUTP nick-end labelling] using the TdT-FragEL DNA fragmentation Kit (Amersham Pharmacia Biotech) and 1 μmol l^{-1} DIG-dUTP. Detection of DIG was carried out by standard procedures using an anti-DIG antibody coupled with alkaline phosphatase and phosphatase reaction with nitroblue tetrazolium chloride (NBT)/5-bromo-4-chloro-3-indolyl phosphate (BCIP) as a substrate.

Results

Induction of apoptosis in interspecies grafts

Apoptosis is used by immune systems of diverse organisms to eliminate foreign and infected cells. We have previously demonstrated an increased level of phagocytosis in *H. vulgaris (=H. attenuata)/H. oligactis* heterografts at the graft site where the epithelia of the two species contact each other (Bosch and David, 1986). To examine whether phagocytosis is preceded in such heterografts by induction of apoptosis, grafts between *H. oligactis* and *H. vulgaris* Basel strain were produced and subjected to several apoptosis detection assays 24 h after grafting. As shown in Fig. 1, we found an increase in the number of apoptotic cells identified by Acridine Orange as well as TUNEL staining in the contact region of heterografts.
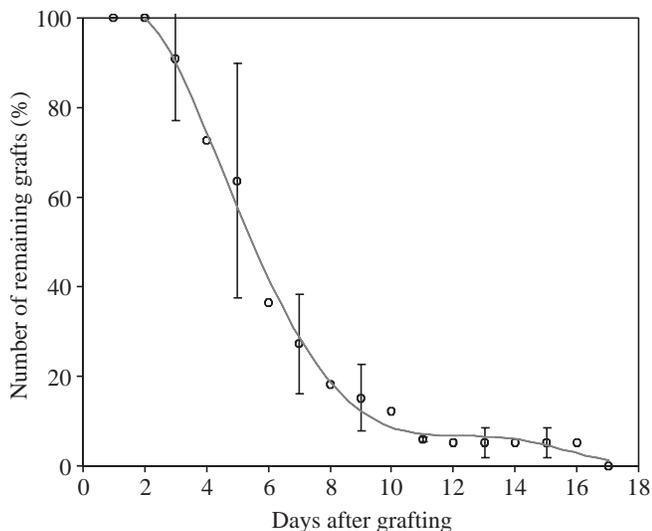

Fig. 2. Stability of *H. vulgaris/H. oligactis* heterografts after grafting. Values are means ± S.D. (*N*=5). The solid line is the polynomial trendline through the data points.
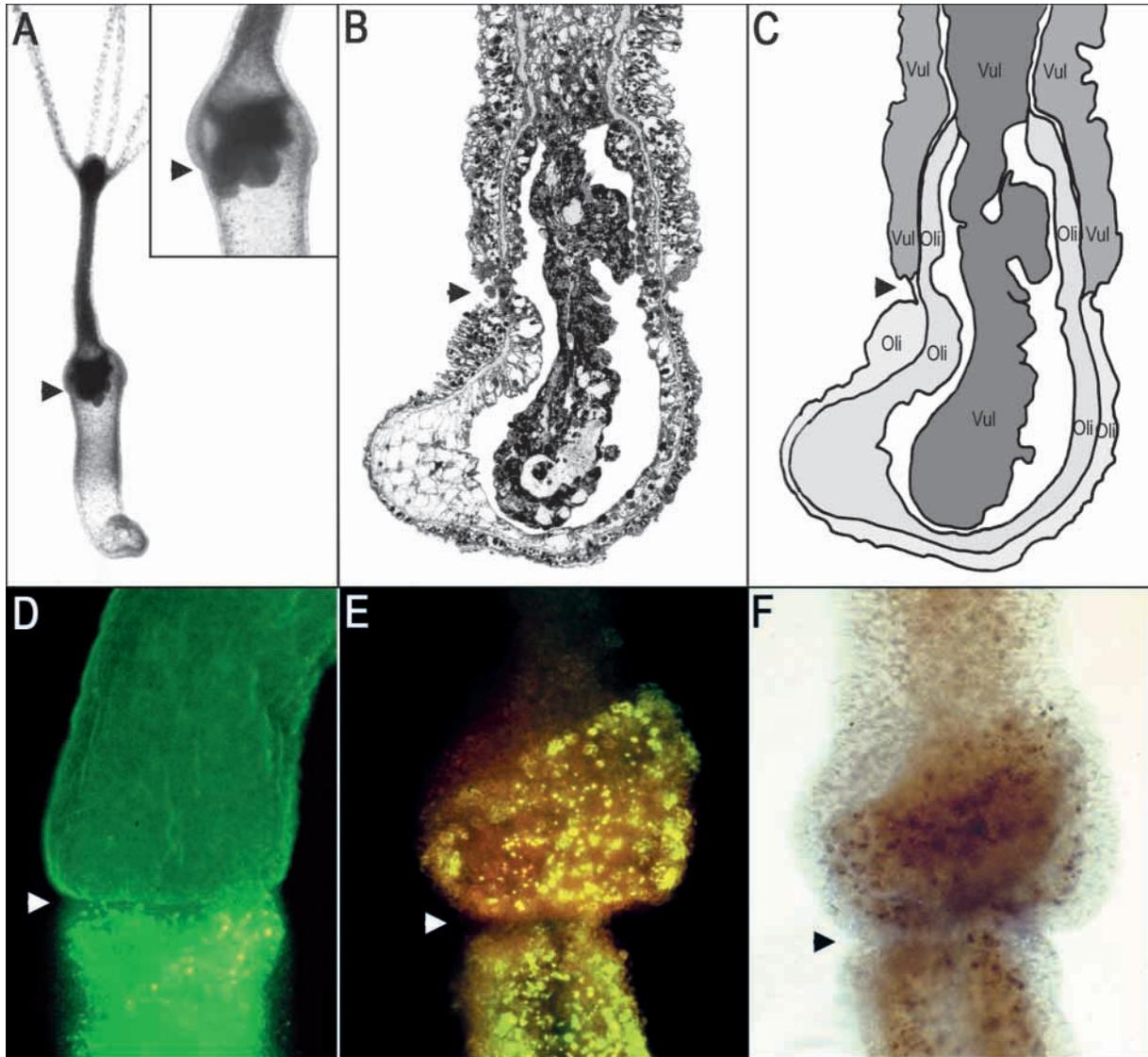
Fig. 3. Displacement of *H. vulgaris* endoderm in heterografts. (A) 1-day-old heterograft with the *H. vulgaris* half placed distally and the *H. oligactis* half proximally. Inset, grafting site at higher magnification. (B) Longitudinal section through a heterograft similar to that shown in A. (C) Schematic representation of the section shown in B. Vul, tissues of *H. vulgaris*; Oli, tissues of *H. oligactis*. Note the clump of *H. vulgaris* endodermal tissue hanging into the gastric cavity. (D,E) *H. vulgaris*/*H. oligactis* heterografts 2 and 24 h after grafting, respectively. *H. oligactis* endoderm is labelled with fluorescent latex beads. (F) Brightfield view of the animal shown in E. Arrowheads indicate heterologous contact in the ectoderm.

Thus, apoptosis is activated when tissues from these two species come in close contact. Interestingly, most of the apoptotic cells were found in the *H. vulgaris* ectodermal epithelium adjacent to the graft border (Fig. 1F–H). Differential accumulation of apoptotic cells in *H. vulgaris* tissue was observed regardless whether *H. vulgaris* was the apical or basal half of the heterograft. This is in agreement with the previous observation that in *H. oligactis*/*H. vulgaris* grafts there is continuous displacement of *H. vulgaris* cells (Bosch and David, 1986), and may indicate that susceptibility to death signals is different in the two species. In control homografts (Fig. 1A,E) only a few apoptotic cells were found scattered along the body axis.

Stability of interspecific grafts

When culturing heterografts we observed that interspecies combinations are not permanently stable (Fig. 2). *H. vulgaris*/*H. oligactis* heterografts were perfectly healed 24 h after transplantation. However, a few days later, a constriction appeared at the graft border of the interspecies combinations and a clump of tissue of unknown origin appeared in the gastric cavity close to the graft junction (Fig. 3A). Within 3 weeks all of the 179 *H. vulgaris*/*H. oligactis* grafts from 5 independent experiments eventually separated, regardless of whether *H. vulgaris* was the apical or the basal partner. In contrast, control *H. vulgaris*/*H. vulgaris* and *H. oligactis*/*H. oligactis* homografts formed permanently stable combinations. Since we
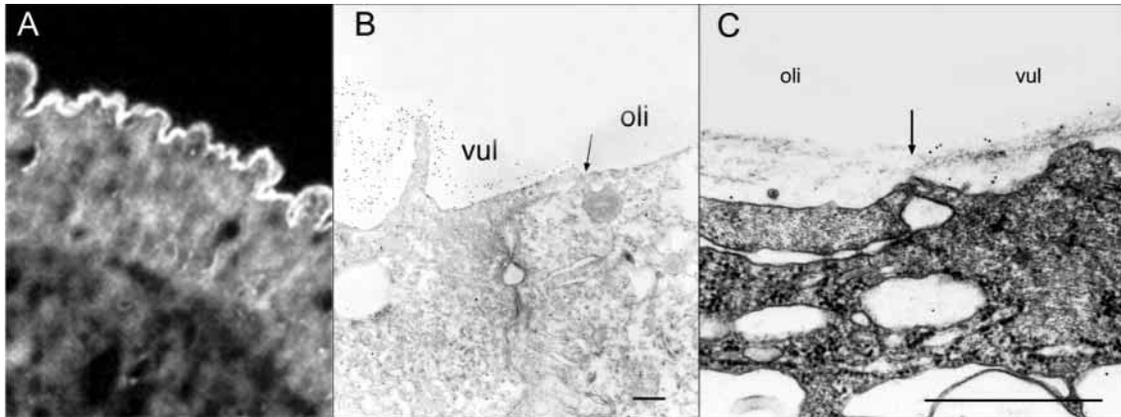
Fig. 4. Species-specific antibodies recognize epithelial cell surface antigens. (A) *H. vulgaris* ectodermal epithelial cells are stained with species-specific antiserum visualized with FITC-labelled secondary antibody. Note the strong staining of the apical surface (glycocalix). (B) Uncontrasted ultrathin section through the *H. vulgaris* (vul)/*H. oligactis* (oli) graft site. Antibodies coupled with 10 nm gold particles mainly stain glycocalyx of *H. vulgaris*. (C) Contrasted ultrathin section through the *H. vulgaris*/*H. oligactis* graft site (same preparation as in Fig. 5) stained with gold-coupled antibodies. Arrows indicate the heterotypic cellular contact. Scale bars, 0.5 μm.

previously observed displacement but no separation of the graft partners (Bosch and David, 1986), these findings stimulated us to study the cellular interactions in more detail.

Differential epithelial movement and displacement of H. vulgaris *endoderm*

To investigate the nature of the amorphous tissue material accumulating at the graft site, we performed detailed histological analysis. As shown in Fig. 3B, the tissue clump corresponds to endodermal tissue of *H. vulgaris* detached from the mesoglea. This indicated that *H. oligactis* endoderm displaces the endoderm of *H. vulgaris* at the graft site. To assess the dynamics of this tissue displacement, we differentially labelled the endoderm of *H. oligactis* using fluorescent beads. Fig. 3D shows such a interspecies combination 2 h after grafting with a sharp boundary between unlabeled *H. vulgaris* and labeled *H. oligactis* endoderm. 22 h later fluorescently labelled *H. oligactis* endoderm is found in the *H. vulgaris* portion of the graft (Fig. 3E). Thus, endoderm of *H. oligactis* underwent rapid movement and displaced *H. vulgaris* endoderm. Similar tissue movement and displacement of *H. vulgaris* endoderm could be observed regardless of whether *H. vulgaris* was the apical or basal partner of the heterograft. In control homografts, neither overlapping nor tissue displacement was observed 24 h after grafting (data not shown). The displacement of *H. vulgaris* endoderm by *H. oligactis* endoderm leads to the overlap of two heterogeneous tissue layers at the graft junction (see Fig. 3C). It seems possible that this overlap contributes to the observed local induction of apoptosis at the graft site.

Impaired cell–matrix and cell–cell contact at the graft site

To investigate the role of cell adhesiveness in the observed instability of the heterografts, cell contacts at the graft site were analysed at the ultrastructural level. The graft junction of 1-day-old *H. vulgaris/H. oligactis* heterografts was identified by both *H. vulgaris*-specific antiserum binding to the apical surface of ectodermal epithelial cells (Fig. 4; see Materials and methods) and the morphology of the glycocalyx, which is thin in *H. vulgaris* and thick in *H. oligactis* (Figs 4 and 5). As shown in Fig. 5C, heterotypic cell contact between an ectodermal epithelial cell of *H. oligactis* and of *H. vulgaris* is characterized by scattered septae-like elements in the places of desmosomes with the spaces between cell membranes being highly irregular (Fig. 5C). In comparison, epithelial contacts in intraspecies grafts are characterized by well developed septae and regular spaces between cell membranes (Fig. 5B,D). These observations suggest that instability of the *H. vulgaris/H. oligactis* heterografts might be caused by impaired cell–cell contact between heterogeneous ectodermal epithelial cells.

The observed overlap of *H. vulgaris* endoderm and *H. oligactis* ectoderm at the graft site (Fig. 3B,C) raises the question of the nature and structure of the extracellular matrix separating the two cell layers. To assess this, we studied the extracellular matrix in 24 h old heterografts by electron microscopy. As shown in Fig. 6, distinct morphological differences were observed between the mesoglea at the graft site and outside of it. At the graft site the mesoglea was highly irregular in shape and structure; in some places it appeared as a thick layer (Fig. 6C,F) while in other regions it was flattened (Fig. 6D,E) or even fragmented (Fig. 6C,E). The mesoglea outside the graft region (Fig. 6C,G) was well constituted and showed a structure and thickness similar to that known in normal polyps.

Development of heteroaggregates

To monitor cell behavior during heterogenous contact more quantitatively, we used a cell aggregation assay. Polyps of *H. vulgaris* and *H. oligactis* were dissociated into cell suspensions, combined and aggregated by centrifugation. The
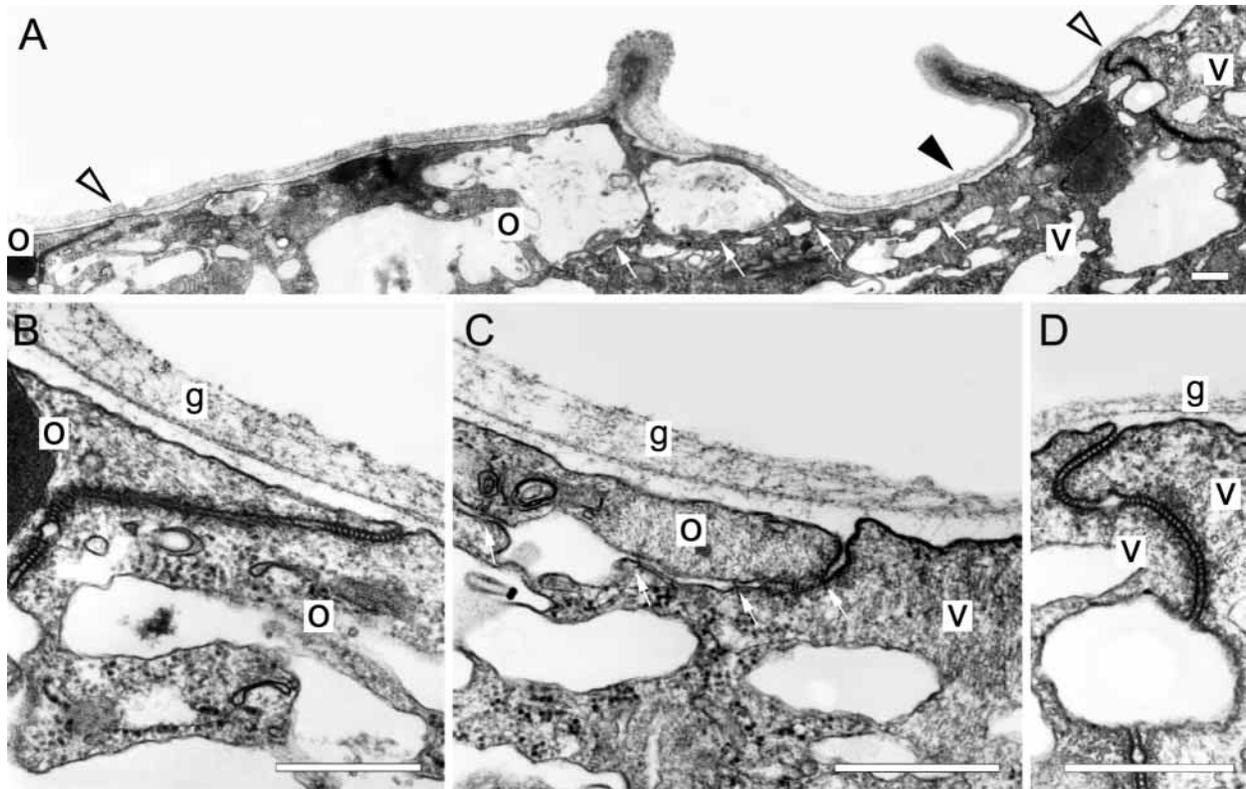
Fig. 5. Ultrastructure of homotypic and heterotypic septate junctions in the ectoderm of a heterograft (24 h old). (A) The left and the right white arrowheads show homotypic junctions between two cells of *H. oligactis* (o), enlarged in (B), and two cells of *H. vulgaris* (v), enlarged in (D), respectively. Note the regular septae and spaces between cell membranes. (C) Enlargement of the heterotypic junction indicated by the black arrowhead in A. White arrows in A point to the heterotypic contact with a few irregular septae and spaces between cell membranes shown in C. g, glycocalyx. Note the species-specific differences in thickness of glycocalyx. Scale bars, 0.5 μm.

resulting aggregates were allowed to develop. At different stages of development aggregates were fixed, embedded and sectioned. To visualize the behavior of cells in the interspecies mixture, we used immunostaining with species-specific antibodies. As shown in Fig. 7, starting from a homogeneous mixture, reaggregated cells from both species quickly became spatially segregated. That is, ectodermal and endodermal cells sorted into their respective cell layers in a species-specific manner, resulting in the formation of clusters of cells of each species 24 h after reaggregation (Fig. 7C). In agreement with the behavior observed in heterografts, in most cases, *H. vulgaris* ectoderm finally engulfed *H. oligactis* endoderm (Fig. 7D,E). Interspecies cell combinations never remained randomly mixed, indicating that cells from both species differ in the strengths of their adhesion. Moreover, heteroaggregates were severely retarded in their development when compared to homoaggregates (Fig. 7F). Thus, since development in *Hydra* is mainly controlled by epithelial cells (Bosch, 1998), the chimeric overlapping of epithelia of different species may result in impaired transduction of developmental signals.

When culturing *H. oligactis*/*H. vulgaris* heteroaggregates we observed that, in contrast to epithelial cells, cells of the interstitial cell lineage did not sort in a species-specific manner. We used holotrichous isorhiza nematocytes, which differ in their morphology between *H. oligactis* and *H. vulgaris*, as a marker for the interstitial cell lineage. 45 days after reaggregation of interspecies cell mixtures, holotrichous isorhiza nematocytes of the *H. oligactis* type were still found to be homogeneously mixed with those of the *H. vulgaris* type in tentacles of the developing chimaeras (data not shown). Since nematocytes of both species can also be detected in tentacles of interspecies grafts (Fig. 7G–I), this observation supports the view that a discriminative allorecognition system, if present, is rather poorly developed in *Hydra*. This is in agreement with observations presented elsewhere (S. G. Kuznetsov and T. C. G. Bosch, manuscript in preparation) that in intraspecies grafts of various *H. vulgaris* strains, contact to allogeneic tissue did not evoke any response in terms of phagocytosis and elimination of non-self cells.

Discussion

We previously have observed an increased number of epithelial cells containing phagocytic vacuoles at the graft site of *H. attenuata* (= *H. vulgaris*)/*H. oligactis* heterografts. Since labeling experiments indicated that *Hydra* epithelial cells in such heterografts are capable of phagocytizing cells of the opposite species, we concluded that epithelial cells are capable
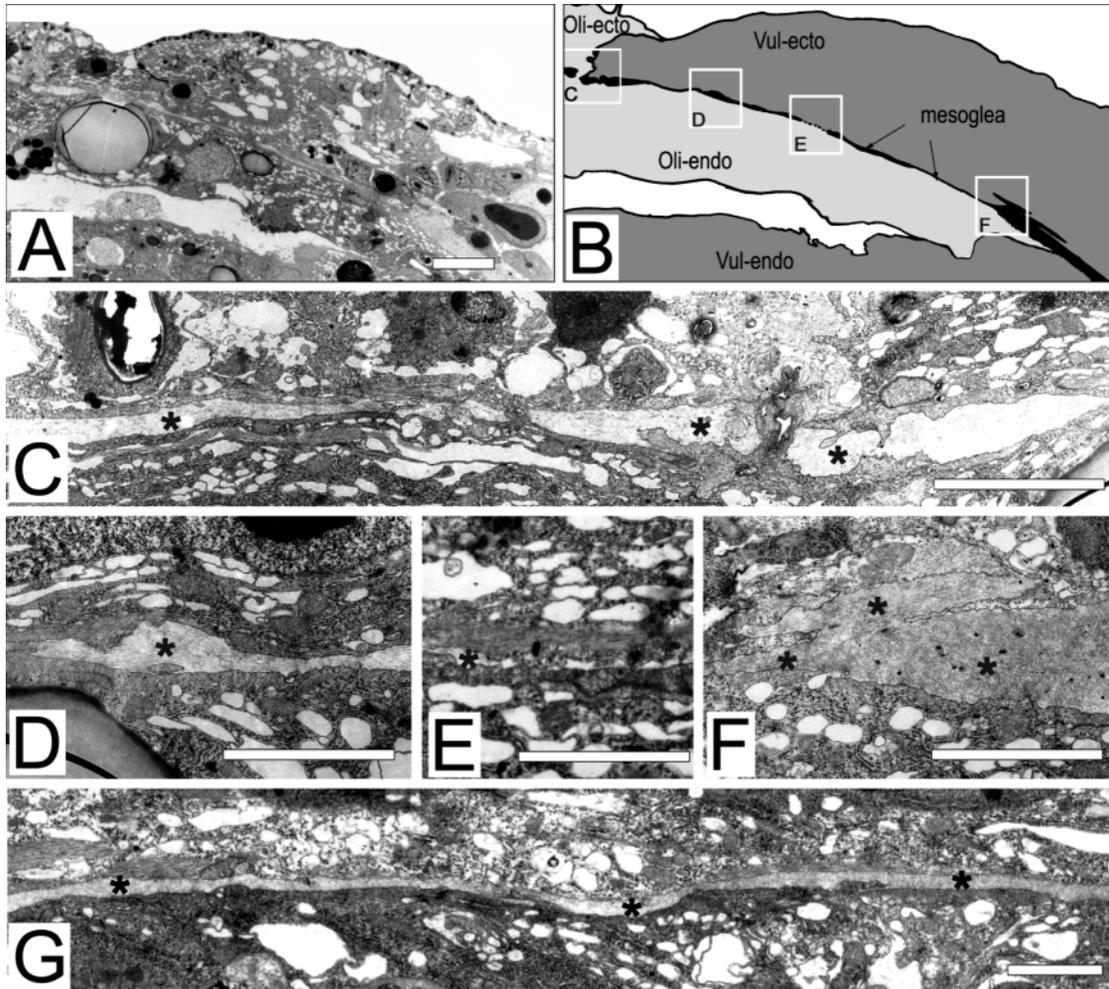
Fig. 6. Ultrastructure of the mesoglea in the chimeric region of a heterograft (24 h old). (A) Overview of the graft site and (B) its schematic representation. Oli-ecto, *H. oligactis* ectoderm; Vul-ecto, *H. vulgaris* ectoderm; Oli-endo, *H. oligactis* endoderm; Vul-endo, *H. vulgaris* endoderm. Frames labelled C–F are enlarged in (C–F), respectively. Note the abnormal morphology of mesoglea (asterisk) in the chimeric region (D–F). (G) Mesoglea in control *H. vulgaris* polyps. Scale bars, 10 μm (A); 5 μm (C–G).

of recognizing non-self and reacting against it (Bosch and David, 1986). An increased interest in the evolutionary ancestry of immune systems and the advent of molecular techniques inspired us to reinvestigate the cell interactions in such interspecies grafts as a prerequisite to directly searching for the molecular mechanisms of this putative discriminative recognition system in *Hydra*.

Apoptosis in interspecies grafts in Hydra

Apoptosis functions as part of a quality control process, eliminating cells that are abnormal, nonfunctional or potentially dangerous to the organisms (Jacobson et al., 1997). In *Hydra* apoptosis has the same morphological features (Bosch and David, 1984, 1986) and uses the same molecular components (Cikala et al., 1999) as in more complex animals. Previous studies have shown that apoptosis in *Hydra* is involved in elimination of 'excess' cells in response to starvation (Bosch and David, 1984; Cikala et al., 1999) as well as in oogenesis (Miller et al., 2000) and spermatogenesis

(Kuznetsov et al., 2001). Recognition and engulfment of apoptotic cells in *Hydra* is carried out by ectodermal epithelial cells, which play an active role as phagocytes (Bosch and David, 1984, 1986; Kuznetsov et al., 2001). The present study with Acridine Orange and TUNEL staining confirmed that the cells phagocytosed by epithelial cells at the graft site underwent apoptosis. Although the sequence of events leading to activation of apoptosis at the graft site remains to be elucidated, we present evidence that the loss of cell anchorage to the extracellular matrix (anoikis) might be a critical step.

Impaired cell–matrix and/or cell–cell contact may lead to induction of programmed cell death

The extracellular matrix separating the two cell layers in *Hydra,* termed mesoglea, contains macromolecules such as laminins, collagens, heparan sulfate proteoglycans and fibronectin-like molecules (Sarras et al., 1991). These molecules play essential roles in cell proliferation, cell migration and morphogenesis (Sarras et al., 1993; Schmid et
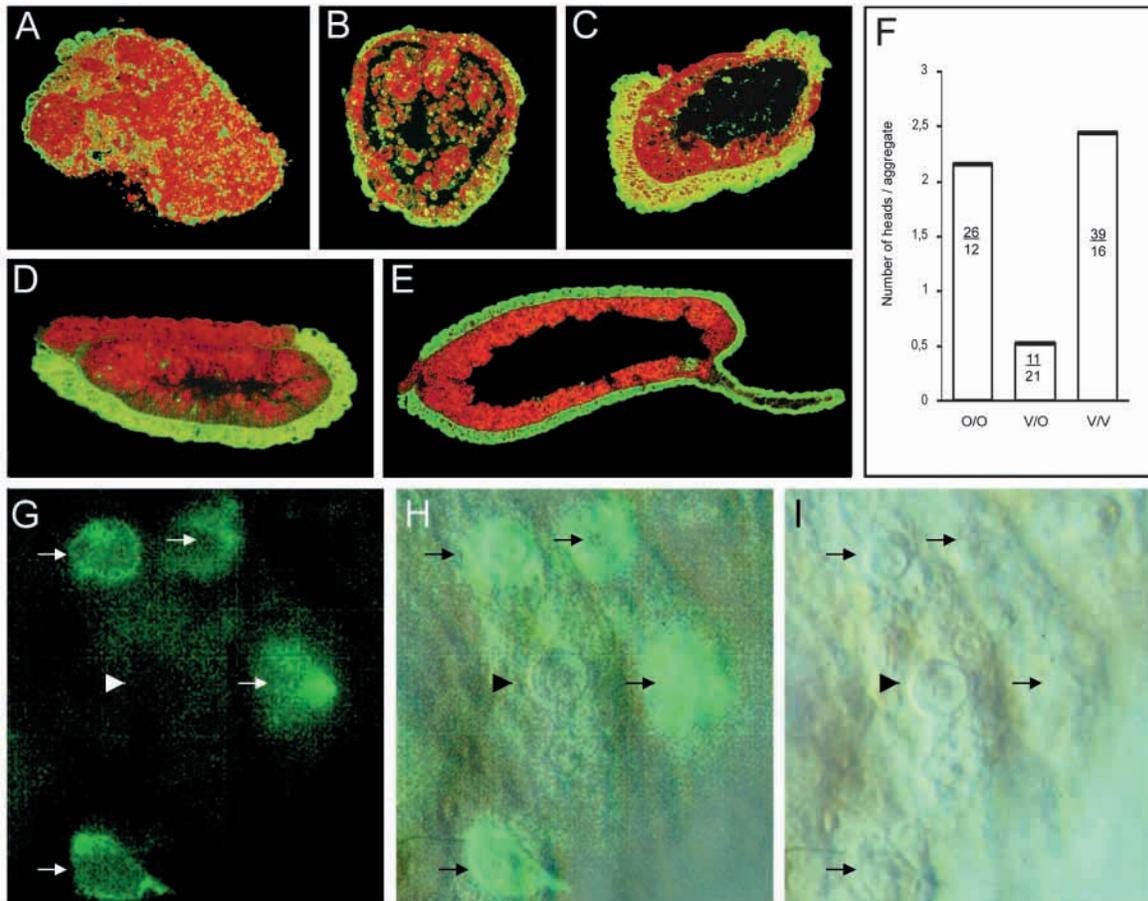
Fig. 7. Heteroaggregates slowly develop to ecto-/endodermal chimeric polyps. (A–E) *H. vulgaris/H. oligactis* heteroaggregates 10 h, 24 h, 7 days, 21 days and 28 days after reaggregation, respectively. *H. vulgaris* cells are stained with species-specific antiserum visualized with FITC-labelled secondary antibody (green). Unlabelled cells of *H. oligactis*, couterstained with Toluidine Blue, appear red. (F) Histogram indicating the number of head structures per aggregate (numbers in columns) on day 7 after reaggregation. o/o and v/v, control homoaggregates from *H. oligactis* or *H. vulgaris* alone, respectively. v/o, heteroaggregates. (G–I) Interstitial cell differentiation products such as nematocytes are not eliminated in heterografts. Shown is the portion of a tentacle of a heterograft (3 days old) stained with an antiserum specific for *H. vulgaris* cells. One *H. oligactis* nematocyte (arrowhead) is embedded next to several *H. vulgaris* nematocytes (arrows).

al., 1999; reviewed in Sarras and Deutzmann, 2001). Curiously, components of *Hydra* mesoglea are differentially synthesized by the two epithelia. *Hydra* laminin participates in formation of basement membranes of both epithelial layers but is secreted exclusively by the endoderm (Sarras et al., 1994). Conversely, collagen-I is synthesized in the ectoderm (Deutzmann et al., 2000). Production of collagen-I by ectodermal epithelial cells is preceded by and dependent on the production of laminin by endodermal epithelial cells (Sarras and Deutzmann, 2001). Our observations indicate that the mesoglea in *H. vulgaris/H. oligactis* heterografts underwent significant changes in shape and structure at the graft site. Thus, incompatible species-specific interactions between certain extracellular matrix molecules may substantially compromise the mesoglea structure and provoke programmed cell death *via* anoikis at the graft site. Taken together, our findings support the idea that the mesoglea separating both cell layers plays a key role in controlling survival of epithelial cells in the basal metazoan *Hydra*. Therefore, it appears that the

dependence of survival of epithelial cells on anchorage to extracellular matrix molecules is an ancient feature of epithelial homeostasis and crucial in metazoan development.

Consistent with this idea, chimeric aggregates showed remarkable retardation of development (Fig. 7F), similar to aggregates in which biosynthesis of mesogleal proteins is perturbed (Sarras et al., 1993). Interestingly, cell interactions in developing heteroaggregates were similar to the one in heterografts and resulted in chimeric polyps consisting of *H. vulgaris* ectoderm and *H. oligactis* endoderm. This cell sorting in heteroaggregates is probably caused by differential cell adhesiveness between the species (Townes and Holtfreter, 1955; Steinberg, 1970; Sato-Maeda et al., 1994). Since substantial signal exchange between ectodermal and endodermal cells is occurring during in *Hydra* morphogenesis (Kishimoto et al., 1996), it seems possible that *H. oligactis* endoderm outcompetes *H. vulgaris* endoderm due to the more effective interaction with the ectodermal layer, even when the ectoderm comprises *H. vulgaris* epithelial cells.

In sum, the present study reveals that interspecific cell contact in *Hydra* initiates apoptosis at the graft site. However, this largely appears to be the result of impaired cell–matrix and cell–cell contact. In contrast to our previous assumption we could not detect any evidence for the ability of *Hydra* epithelial cells to specifically recognize non-self cells.

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