Journal of Cellular Biochemistry 100:1170–1177 (2007)

Rat Mast Cells Communicate With Fibroblasts Via Gap Junction Intercellular Communications

Stephen R. Au,[1] Katherine Au,[2] Gregory C. Saggers,[2] Neel Karne,[2] and H. Paul Ehrlich[2]*

[1]University of California Davis, Davis, CA
[2]Department of Surgery, Division of Plastic Surgery; Hershey Medical Center, Hershey, PA

Abstract Usually mast cells (MCs) modulate other cellular activities through the release of their cytoplasmic granules. Recently, gap junctional intercellular communication (GJIC) between an established human MC cell line (HMC-1) co-cultured with human dermal fibroblasts in fibroblast populated collagen lattices (FPCLs), enhanced the rate and degree of FPCL contraction. However, HMC-1 cells were unable to generate GJIC with human neonatal fibroblasts in monolayer culture. Here freshly isolated rat peritoneal MCs are co-cultured with fibroblasts in collagen lattices and in monolayer culture *in vitro* and introduced into rat polyvinyl alcohol (PVA) sponge implants *in vivo*. Co-cultured MC-FPCL contracted faster and to a greater degree. Loading Calcein AM green fluorescent dye into red fluorescent DiI tagged MC generates MC-paratroopers. When MC-paratroopers form GJIC with fibroblasts, some green dye is passed into the fibroblast, while the MC-paratrooper retains both its red and green fluorescence. MC-paratroopers passed green fluorescent dye into both human and rat dermal fibroblasts in monolayer culture. In rats 7-day-old subcutaneous PVA sponge implants, which received an injection of MC-paratroopers, exhibited auto-fluorescent green fibroblasts, when harvested 24 h later. MC-paratroopers pretreated with a long-acting GJIC inhibitor prior to their introduction into PVA sponge implants, failed to pass dye into fibroblasts. It is proposed that GJIC between granulation tissue fibroblasts and MCs can modulate some aspects of wound repair and fibrosis. J. Cell. Biochem. 100: 1170–1177, 2007.

Key words: rat mast cells; fibroblasts; collagen lattices; PVA sponge implants; granulation tissue

Wound healing proceeds through a co-ordinated progression of overlapping phases, which include lag (inflammation), proliferation (wound fibroblast migration, proliferation and deposition of granulation tissue), and remodeling (granulation tissue maturation into a scar). Hypertrophic scarring or excess fibrosis is speculated to be associated with the mast cell (MC) [Kischer et al., 1978; Hebda et al., 1993; Xu et al., 2002]. The current concept regarding MC influencing wound fibroblasts involves MC degranulation and the release of soluble mediators such as histamine, heparin, cytokines (IL-4, IL-8, TNFα), and/or the proteases, chymase and tryptase [Cairns and Walls, 1997; Gruber et al., 1997; Trautmann et al., 2000; Wierecky et al., 2000; Gibbs et al., 2001]. As an example, the MC proteases, chymase and tryptase, which are reported elevated in excess scarring, promote fibroblast proliferation and collagen synthesis [Gruber et al., 1997; Asano-Kato et al., 2005; Matsushima et al., 2006].

Gap junction intercellular communication (GJIC) allows direct passage of small molecules between the cytoplasm of coupled cells. Molecules of less than 1,200 molecular weight can pass between coupled cells through gap junction channels. Intercellular communication between cells through gap junctions is rapid and can facilitate coordinated cellular responses between coupled cells. Gap junction channels are gated structures, where the channel can be in a closed or open state [Kumar and Gilula, 1996]. The gap junction channel is made up of a pair of plasma membrane embedded structures between neighboring cells, called connexons. The connexon is composed of connexin (Cx) proteins [Musil and Goodenough, 1991]. A pair of hexameric oligomer protein connexons, anchored in the plasma membrane of neighboring cells, comes together to form the gap junction channel. There are numerous Cx isoforms with Cx-43 the major Cx of

*Correspondence to: Dr. H. Paul Ehrlich, 500 University Dr. Hershey, PA 17033. E-mail: pehrlich@psu.edu
Received 20 May 2006; Accepted 14 July 2006
DOI 10.1002/jcb.21107

fibroblasts. During wound re-epithelialization, changes in GJIC are reported in keratinocytes. Keratinocytes in intact epidermis are coupled through gap junction channels made up with CX-43 [Lampe et al., 1998]. After wounding keratinocytes at a wound edge fail to express CX-43 and lack GJIC [Kretz et al., 2003; Brandner et al., 2004]. The loss of GJIC and Cx-43 expression is associated with migrating keratinocytes. At the termination of keratinocyte migration and keratinocyte maturation, keratinocytes return to their normal stationary state, the expression of CX-43 returns and GJIC is restored between cells. Commonly, GJIC involves the passage of small molecules between like-cells, such as fibroblast-to-fibroblast or keratinocyte-to-keratinocyte; however, GJIC can occur between heterotypic cell populations, such as between MCs and fibroblasts [Oliani et al., 1995].

Dermal fibroblasts in skin establish a network of coordinated cellular communications through GJIC [Salomon et al., 1988]. Blocking GJIC with uncouplers in developing granulation tissue reduces the amount of granulation tissue deposition [Ehrlich and Diez, 2003]. The in vitro fibroblast populated collagen lattice (FPCL) contraction model facilitates the study of interactions between fibroblasts and collagen [Bell et al., 1979]. The co-culture of MCs with fibroblasts in the manufacture of FPCLs enhances FPCL contraction [Yamamoto et al., 2000; Skold et al., 2001]. Numerous proposed mechanisms for the enhancement of lattice contraction include the release of tryptase [Trautmann et al., 1998], chymase [Nishikori et al., 1998], and/or histamine [Garbuzenko et al., 2002] as well as stem cell factor (SCF)/c-kit interactions between fibroblasts and MCs [Yamamoto et al., 2000]. Another proposed mechanism for enhanced co-cultured MC-FPLC contraction is the establishment of GJIC between MC and fibroblast cell lines in a lattice contraction model. The enhancement of co-cultured human MC-FPCL contraction is shown to involve GJIC between MCs and fibroblasts [Moyer et al., 2004].

In granulation tissue does GJIC between MCs and fibroblasts occur? The generation of GJIC between freshly isolated rat peritoneal MCs with fibroblasts in cell culture, in collagen lattices, and in granulation tissue is presented. When human neonatal dermal fibroblasts are co-cultured with an established MC cell line (HMC-1) in a collagen lattice, heterogeneous GJIC forms between fibroblasts and MCs. However, these same MCs are unable to make heterogeneous GJIC with the same human neonatal dermal fibroblasts in monolayer culture. Here we examine the possibility that freshly isolated rat peritoneal MC will form heterogeneous GJIC with fibroblasts in monolayer culture, in granulation tissue and promote co-cultured MC-FPCL contraction.

METHODS

Cell Cultures

Primary human neonatal foreskin fibroblasts were maintained in monolayer culture with biweekly changes with Dulbecco's modification of Eagles medium (DMEM) supplemented with gentamicin, 10 μg/ml, and 10% fetal bovine serum (FBS), referred to as complete DMEM. When cells became confluent, they were passed 1:1. The human fibroblasts were studied in their 8th–10th passage. HMC-1, a human transformed MC cell line, was a generous gift from Dr. Butterfield. It was maintained in Iscove's medium supplemented with 10% FBS [Butterfield et al., 1988].

To obtain rat wound fibroblasts a polyvinyl alcohol (PVA) sponge (1.2×0.3 cm thick disks) was placed in subcutaneous pockets in an adult rat as will be described below. At 7 days the sponge was surgically removed and cut into 1 mm pieces, which were placed in 35 mm tissue culture dishes, covered with a glass coverslip, and given 1 ml of complete DMEM. Rat fibroblasts that grew out from explants were maintained in complete DMEM and they were studied at their 6th passage.

FPCL Contraction

Either rat fibroblasts or human neonatal fibroblasts at 2.5×10^4 cells were combined with 2.4×10^4 freshly isolated rat peritoneal MCs. The cells were mixed with 0.625 mg of rat tail tendon acid-soluble collagen in complete DMEM in a final volume of 0.5 ml. The cell-collagen-medium mixture was placed in a separate well of a 24-well cluster plate, which was transferred to a 37°C incubator, where the collagen polymerized in less than 90 s [Ehrlich et al., 2000]. The lattices were freed from the wells 30 min after casting with a glass rod. The diameter of each lattice was measured daily and the calculated areas recorded.

Statistical significance was based upon one-way ANOVA analysis, where a P-value <0.05 was the measure of significance.

Rat PVA Sponge Studies

Polyvinyl alcohol sponge doughnut discs 1.2 cm in diameter and 0.3 cm thick with a central hole 0.5 cm in diameter were manufactured with a thin polycarbonate plastic 1.2 cm diameter disc secured with silastic glue to their bottom surface. The PVA sponge doughnut's central reservoir served as the compartment for injecting MC-paratroopers into the implants. Three Sprague-Dawley male rats (each 300 g) were anesthetized by halothane inhalation. Each rat's dorsum was clipped and cleaned with 70% alcohol. A 2 cm incision was made with a scalpel over the dorsal midline, and subcutaneous pockets were created on each side employing blunt nose scissors. The pockets were located 3 cm caudal to the forelimbs in a transverse plane. The PVA sponge doughnuts were boiled in water for 5 min, blotted to remove excess water, then a single PVA doughnut was inserted into each subcutaneous pocket with its plastic base resting on the muscle fascia and the PVA sponge abutting the overlying dermal layer. After the implantation, the incision was closed with stainless steel staples and each rat returned to his cage upon recovering from anesthesia.

MC Harvesting

Sprague-Dawley rats 300–350 g were anesthetized with halothane, the skin over the abdomen clipped, and cleaned with 70% alcohol. A 2 cm incision was made with a scalpel over the ventral midline passing into the peritoneum. A Blake 7 mm flat full fluted silicone drain (Ethicon, Inc., Somerville, NJ) was inserted into the abdominal cavity and attached to Jackson-Pratt bulb suction. Normal saline (NS) with 10% FBS, which had a total volume of 50 ml, was injected into the abdominal cavity via blunt syringe and recollected by bulb suction. The collected peritoneal wash was centrifuged at 1,000*g* for 5 min and the supernatant discarded. The pellet was resuspended in Iscove's medium (BioWhittaker, Walkerville, MD) supplemented with 10% FBS. Employing the Accudenz density gradient (Accurate Chemical and Scientific Corporation, Westbury, NY) MCs were isolated using the supplier's directions for mononuclear cell isolation. A gradient of 27.8–20% Accudenz Sucrose diluent was used for fractionation. Confirmation of MC was documented by Toluidine Blue cellular staining.

MC-Paratroopers

Utilizing an adapted protocol MC-paratroopers were generated by suspending a pellet of freshly isolated rat peritoneal MC in 1.0 ml of 300 mM glucose with 30 mM HEPES buffer [Goldberg et al., 1995; Moyer et al., 2004]. The MC suspension received 10 μl of Calcein AM (1 mg/ml) in DMSO and 8 μl of 1,1′-dihexadecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate (DiI) 2.5 mg/ml in 70% ethanol (Molecular Probes, Eugene, OR). The MC suspension was incubated for 10 min at 37°C, pelleted by centrifugation, the pellet washed two times with PBS, the cells resuspended in 1 ml of Iscove's medium with 10% FBS and then the cell number determined with a hemacytometer. The dye-loaded, labeled rat peritoneal MCs were referred to as MC-paratroopers. The MC-paratroopers had plasma membranes permanently stained fluorescently red by DiI and their cytoplasm loaded with soluble fluorescent green Calcein AM. When a paratrooper formed gap junctions with other cells, green Calcein AM dye was passed into the coupled cells, and the paratroopers retained their red fluorescence. Paratroopers contained red and green fluorescence, while a coupled cell contained only green fluorescence. Suspended MC-paratroopers were cast with fibroblasts in MC-FPCLs, placed on fibroblasts in monolayer or injected into PVA sponge implants.

GJIC Inhibition

Rat peritoneal isolated MCs were suspended in 1 ml Iscove's medium supplemented with 10% FBS in a 12 ml centrifuge tube. The MC suspension received 1.2 nM of trifluoromethyl ketone fatty acid amide hydrolase (FAAH), a long-acting uncoupler, a generous gift of Dr. Dale L. Boger (Scripps Research Institute, La Jolla, CA) [Guan et al., 1997; Boger et al., 1998]. The dishes were then returned to the incubator for 30 min. The MCs were incubated with the uncoupler for 3 h at 37°C, pelleted by centrifugation, washed two times with PBS, and then prepared as fluorescent MC-paratroopers as described above. The FAAH-treated paratroopers, FAAFA medium, were resuspended in 1 ml of fresh Iscove's medium supplemented with 10% FBS.

MC-Paratroopers in PVA Implants

On post day 7 of PVA implantation, MCs were isolated from the peritoneal cavity and paratrooper generated. The rat was anesthetized with halothane, the skin over the PVA implant cleaned with 70% alcohol, and 0.15 ml of wound fluid was removed from each PVA implant central reservoir using a 1 ml syringe with a 22-gauge needle attached. Following the removal of wound fluid, 0.15 ml of either a suspension of intact MC-paratroopers, or FAAH inhibitor treated MC-paratroopers was injected into the central reservoir of the PVA sponge. After 24 h, the rats were sacrificed by an IP injection of Sodium Pentobarbital and the two PVA sponges with their subcutaneous capsules were excised with sharp dissection and divided into two halves. One of the halves was fixed in 4% paraformaldehyde (PFA) for 30 min at 4°C, then transferred to PBS, embedded in OCT Compound (Sakura Finetech; Torrance, CA), frozen and stored at −80°C until cryosectioned. Frozen sections, 10 μm in thickness, were cut, fixed in 4% PFA for 5 min, and then rinsed in PBS. Cell nuclei were stained with 4′6-diamidine-2 phenylindole, dihydrochloride (DAPI) (Molecular Probes, Inc.), where 3 μl of 100 μM DAPI was diluted in 2 ml of PBS. The sections were stained for 5 min at room temperature, mounted in PBS:Glycerol (9:1) then covered with a glass coverslip. An inverted fluorescence Zeiss microscope was used to examine sections. Appropriate filters were employed to identify DiI (red), DAPI (blue), and Calcein AM (green) cells. Labeled cells were recorded using a Photometrics Cool Snap Digital camera with a software package supplied by Biovision (Arlington, VA).

RESULTS

To confirm the isolated rat peritoneal cells were MCs, a 10 μl suspension of the MC preparation was smeared on a glass slide, the slide fixed and stained with Toluidine Blue. More than 90% of the cells were MCs.

FPCL Co-Cultured With MCs

Mast cell co-cultured FPCLs were cast and changes in lattice size, lattice contraction, were followed for 2 days. As shown in Table I the contraction, the reduction in lattice area, of the co-cultured MC-FPCL was greater than FPCL without MC. Lattices made with only MC, MC-populated collagen lattices failed to show any lattice contraction over the 2-day period. The inclusion of MCs with fibroblasts in the casting of co-cultured MC-FPCLs increased the rate and degree of lattice contraction from 56% decrease in size to a 75% decrease in size at day 1 and from 65% to 80% on day 2. The co-cultured MC-FPCL enhancement of lattice contraction was statistically significant ($P < 0.01$). It is clear that MCs do not directly contribute to lattice contraction; rather it appears MCs promote fibroblasts to escalate their capacity to contract collagen lattices.

Fibroblasts in Monolayer Plus Rat MC-Paratroopers

A suspension of MC-paratroopers was layered on top of a confluent monolayer of primary human dermal fibroblasts. After 2 h incubation the co-cultured cells were viewed with a fluorescent microscope. Both double tagged fluorescent rat MC-paratroopers and autofluorescent green human dermal fibroblasts were identified (see Fig. 1A). The mono-fluorescent fibroblasts in monolayer were generated by the passage of Calcein AM dye from the MC-paratroopers into fibroblasts. Unlike HMC-1 MCs, these rat MCs were able to pass Calcein AM dye directly into fibroblasts in monolayer through gap junction channels.

TABLE I. Co-Culture MC-FPCL Contraction

	Day 1	Area change	Day 2	Area change
MC alone	201 ± 0 mm^2	(0%)	201 ± 0 mm^2	(0%)
Fibroblasts alone	89 ± 8 mm^2	(56%)	71 ± 8 mm^2	(65%)
Combination	50 ± 5 mm^2	(75%)	41 ± 3 mm^2	(80%)

*$P \leq 0.01$ using.
FPCL were cast with only rat MC, only human dermal fibroblasts or a combination of MC and human dermal fibroblasts. There were 6 lattices in each group and their sizes were recorded daily.

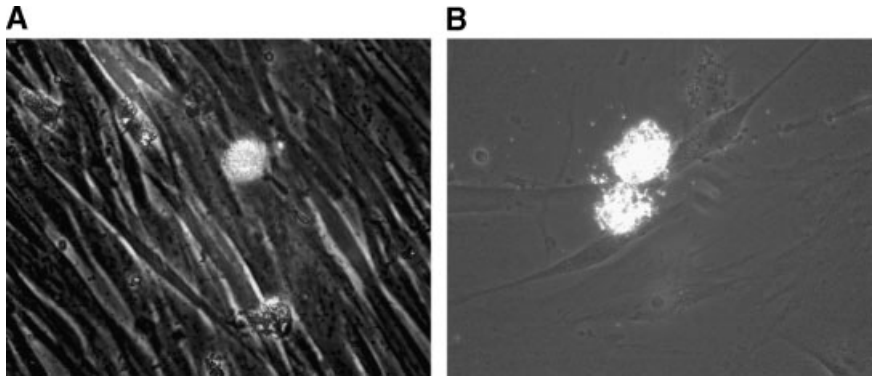

Fig. 1. Prelabeled rat peritoneal mast cell paratroopers were layered on a monolayer of human dermal fibroblasts and viewed 4 hours later. Panel A shows a round mast cell paratrooper (DiI-tagged and Calcein AM dye loaded) that has passed dye into fibroblasts beneath it (magnification 20×). In panel B two round mast cell paratroopers (DiI-tagged and Calcein AM dye loaded) that were pretreated with FAAH are viewed with a rat fibroblast layer beneath them (magnification, 40×). The Calcein AM dye was not passed into the three fibroblasts beneath these paratroopers.

Rat MC-Paratroopers in PVA Sponge Implants

Gap junctional intercellular communication between MC-paratroopers and fibroblasts contained in granulation tissue was investigated. MC-paratroopers were injected into 7-day-old PVA sponge implants and the implants harvested 1 day later. Within PVA sponge granulation tissue MC-paratroopers were identified by their spherical shape and their duel green and red fluorescence. Figure 2A shows two dual tagged fluorescent DiI and Calcein AM, which appeared Yellow-red, tagged MC-paratroopers. These paratroopers were associated with a number of mono-fluorescent green fibroblasts. The Calcein AM dye was passed into fibroblasts from newly arrived paratroopers. To confirm the identity of MC-paratroopers in the granulation tissue, the green filter was removed from Figure 2A, and shown in Figure 2B, the red fluorescent MCs are easily identified. All nuclei were fluorescently blue stained, which demonstrated the nuclei of both MC-paratroopers and fibroblasts in granulation tissue.

FAAH Blocked MC-Paratroopers

Mast cell paratroopers were plated on top of a monolayer of rat fibroblasts and viewed 4 h later. Figure 1B shows a pair of fluorescent MC-paratroopers with three non-fluorescent fibroblasts beneath them. No green Calcein AM fluorescent dye was passed from these FAAH-

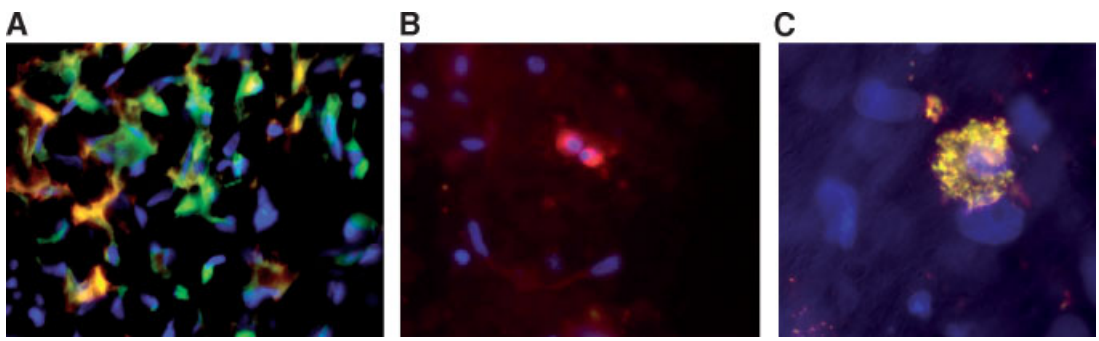

Fig. 2. Prelabeled peritoneal mast cell paratroopers were injected into a PVA sponge implant. Sponges were harvested at 24 hours, cryosections cut and viewed with a fluorescent microscope, which distinguished red and green fluorescence specific for DiI and Calcein AM, as well as a blue fluoresence specific for DAPI. In panel A a pair of pre-labeled peritoneal mast cell paratroopers, shown as yellow-red cells had passed green dye into numerous neighboring fibroblasts within the PVA sponge implant (magnification, 20×). Panel B is the same area as panel A with only the red and blue filters, where the nuclei of mast cells and fibroblasts are shown in blue and the mast cell paratroopers in red (magnification, 10×). Panel C is a cryosection from a PVA sponge injected with MC paratroopers pretreated with the uncoupler agent FAAH. The yellow and green fluorescent mast cell has a spherical shape and no dye has been passed to the surrounding fibroblasts with blue stained nuclei (magnification, 60×).

pretreated MC-paratroopers. Figure 2C is a fluorescence microscopy from a PVA sponge implant that received MC-paratroopers that were pulsed for 3 h with FAAH inhibitor prior to their introduction into the sponge. FAAH inhibitor, which is a long-acting GJIC inhibitor, blocked MC-paratroopers from passing their Calcein AM dye into fibroblasts. Only red and green, which appeared as yellow-red fluorescence, MC-paratroopers were identified in these PVA sponges. MC-paratroopers that were pretreated with FAAH inhibitor failed to develop GJIC with fibroblasts in rat PVA sponge implants.

DISCUSSION

Mast cells are implicated in hypertrophic scarring and keloids [Kischer and Bailey, 1972; Smith et al., 1987].

Increase in rat wound breaking strength is associated with the local accumulation of MCs at the healing incision wound site [Sasaki et al., 2003]. Wound repair and scarring require coordinated cellular functions, as an example, fibroblasts must initially migrate into the wound site, become stationary and then synthesize components of a new connective tissue matrix. If, for example, fibroblasts became prematurely stationary and failed to migrate into the wound site, wound healing would be compromised. It is proposed that MCs form heterogeneous gap junctions with fibroblasts within granulation tissue, which influence some of the activities of fibroblasts in repair and scarring.

When casting FPCL with human dermal fibroblasts, the inclusion of an established MC line, HMC-1 cells, enhances lattice contraction. That enhancement of lattice contraction requires heterotypic GJIC between fibroblasts and HMC-1 cells [Moyer et al., 2004]. However, HMC-1 cells are unable to form GJIC with fibroblasts maintained in monolayer culture. HMC-1 cells like other established MC cell lines have numerous deficiencies compared to endogenous MC [Drexler and MacLeod, 2003]. As an example HMC-1 cells lack chymase and heparin [Drexler and MacLeod, 2003] and they grow in cell culture without the inclusion of cytokines [Butterfield et al., 1988]. HMC-1 cells are defective in making GJIC with neonatal fibroblasts in monolayer, but readily make GJIC with fibroblasts suspended in a collagen lattice. The ability of HMC-1 cells to promote neonatal fibroblast proliferation is a feature of some primary fibroblast cell lines but other cell lines were unresponsive [Trautmann et al., 1998]. Freshly isolated rat peritoneal MCs form GJIC with both human neonatal and rat fibroblasts maintained in monolayer. Like HMC-1 when suspended in collagen lattices, they enhance lattice contraction. One possibility is fibroblasts in monolayer have distinct surfaces: a ventral surface; where focal adhesions develop, linking the cell to the culture dish surface; lateral edges that form contacts with neighboring cells and a dorsal surface that is in contact with culture medium. The expectation is that connexons would reside in the plasma membrane at the edge of fibroblasts and be absent from the cell's ventral or dorsal surfaces. Fibroblasts suspended in a three-dimensional collagen lattices do not have distinct ventral or dorsal zones. Fibroblasts in collagen matrices should be uniform in the distribution of cell surface receptors and other plasma membrane proteins. The speculation is that the initial binding of HMC-1 cells to the dorsal surface of fibroblasts in monolayer requires specific receptors that are absent on some fibroblast surfaces [Trautmann et al., 1998]. Unlike the HMC-1 cells, freshly isolated rat peritoneal MCs have cell surface receptors that initially bind to the dorsal surface of primary rat and human fibroblasts in monolayer. These receptors initiate the initial interactions between the plasma membrane connexons from a closely linked MC and fibroblast.

Like HMC-1 freshly isolated rat peritoneal MCs promote the contraction of co-cultured MC-FPCL. The identity of molecule(s), which pass between MCs and fibroblasts within collagen matrices responsible for promoting lattice contraction, is not known. The molecule(s) is required to have a molecular weight of less 1,200 so it can pass through gap junction channels. The size restriction limits the number of MC candidates that may enhance lattice contraction. Possibilities include secondary messages like Ca^{2+}, cAMP, fatty acids, or small peptides. Proteins and polysaccharides are too large to pass through these channels. It is most likely that the passage of molecules is from the MC to the fibroblast, rather than from the fibroblast to the MC. GJIC between fibroblasts enhances lattice contraction [Ehrlich et al., 2000]. One possibility is that MC forming heterogeneous GJIC with fibroblasts may

increase the density of gap junctional channels that form between fibroblasts.

Freshly isolated rat peritoneal MCs make heterogeneous GJIC with wound fibroblasts in granulation tissue. GJIC usually occurs between homogeneous cell types, such as fibroblast to fibroblast. GJIC between heterogeneous cell types is less commonly reported. In vitro MC-paratroopers pass their dye into fibroblasts that are suspended in a collagen lattice, which demonstrate direct communications between MCs and fibroblasts through gap junction channels. In vivo MCs form heterogeneous gap junction channels with wound fibroblasts in rat granulation tissue. Based upon preliminary histological evaluations, it is not obvious what effect MC-fibroblast-GJIC has on granulation tissue deposition. The introduction of gap junction channel uncouplers into PVA sponge implants alters the histological character of the granulation tissue deposited within those treated implants [Ehrlich and Diez, 2003]. The daily injection of uncouplers (heptanol and endosulfan) for 7 days increases fibroblast density in the surrounding capsule and cavities within the sponge. By polarized light microscopy the birefringence intensity of the newly deposited collagen fiber bundles within an uncoupler-treated PVA sponge implant is reduced compared to an untreated PVA sponge implant. Cell culture experiments report that blocking GJIC with uncouplers reduces collagen synthesis [Ehrlich et al., 2006]. Collagen synthesis is inhibited at the translational level within uncoupler-treated fibroblasts. It is proposed that in the repair process the progression of coordinated phenotypic changes in fibroblasts requires GJIC. As an example, when PVA sponge implants are locally injected with GJIC uncouplers, the transformation of fibroblasts into myofibroblasts is retarded [Ehrlich and Diez, 2003]. The findings from the experiments presented here open up the possibility that GJIC between MCs and fibroblasts may influence the quantity and quality of deposited granulation tissue. In normal dermis through GJIC, fibroblasts reside in a network of coupled cells [Salomon et al., 1988] and fibroblasts may reside in a network of coupled cells in granulation tissue as well. A single MC forming a GJIC with a fibroblast, which is a member of a coupled network of cells, has the potential to modulate the activity of that entire network of coupled fibroblasts. Hence a few MCs may modulate a much greater number of fibroblasts within a network of coupled cells.

REFERENCES

Asano-Kato N, Fukagawa K, Okada N, Dogru M, Tsubota K, Fujishima H. 2005. Tryptase increases proliferative activity of human conjunctival fibroblasts through protease-activated receptor-2. Invest Ophthalmol Vis Sci 46:4622–4626.

Bell E, Ivarsson B, Merrill C. 1979. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. Proc Nat Acad Sci (USA) 76:1274–1278.

Boger DL, Patterson JE, Guan X, Cravatt BF, Lerner RA, Gilula NB. 1998. Chemical requirements for inhibition of gap junction communication by the biologically active lipid oleamide. Proc Natl Acad Sci USA 95:4810–4815.

Brandner JM, Houdek P, Husing B, Kaiser C, Moll I. 2004. Connexins 26, 30, and 43: Differences among spontaneous, chronic, and accelerated human wound healing. J Invest Dermatol 122:1310–1320.

Butterfield JH, Weiler D, Dewald G, Gleich GJ. 1988. Establishment of an immature mast cell line from a patient with mast cell leukemia. Leuk Res 12:345–355.

Cairns JA, Walls AF. 1997. Mast cell tryptase stimulates the synthesis of type I collagen in human lung fibroblasts. J Clin Invest 99:1313–1321.

Drexler HG, MacLeod RA. 2003. Leukemia-lymphoma cell lines as model systems for hematopoietic research. Ann Med 35:404–412

Drexler HG, MacLeod RAF. 2003. Editorial: Malignant hemoatopoietic cell lines: In vitro models for the study of mast cell leukemia. Leuk Res 27:671–676.

Ehrlich HP, Diez T. 2003. A role for gap junctional intercellular communications in wound repair. Wound Repair Regen 11:481–489.

Ehrlich HP, Gabbiani G, Meda P. 2000. Cell coupling, CX 43 expression and fibroblast populated collagen lattice contraction. J Cell Physiol 184:86–92.

Ehrlich HP, Sun B, Saggers GC, Kromath F. 2006. Gap junction communications influence upon fibroblast synthesis of type I collagen and fibronectin. J Cell Biochem 98:735–743.

Garbuzenko E, Nagler A, Pickholtz D, Gillery P, Reich R, Maquart FX, Levi-Schaffer F. 2002. Human mast cells stimulate fibroblast proliferation, collagen synthesis and lattice contraction: A direct role for mast cells in skin fibrosis. Clin Exp Allergy 32:237–246.

Gibbs BF, Wierecky J, Welker P, Henz BM, Wolff HH, Grabbe J. 2001. Human skin mast cells rapidly release preformed and newly generated TNF- alpha and IL-8 following stimulation with anti-IgE and other secretagogues. Exp Dermatol 10:312–320.

Goldberg GS, Bechberger JF, Naus CC. 1995. A preloading method of evaluating gap junctional communication by fluorescent dye transfer. Biotechniques 18:490–497.

Gruber BL, Kew RR, Jelaska A, Marchese MJ, Garlick J, Ren S, Schwartz LB, Korn JH. 1997. Human mast cells activate fibroblasts: Tryptase is a fibrogenic factor stimulating collagen messenger ribonucleic acid synthesis and fibroblast chemotaxis. J Immunol 158:2310–2317.

Guan X, Cravatt BF, Ehring GR, Hall JE, Boger DL, Lerner RA, Gilula NB. 1997. The sleep-inducing lipid oleamide deconvolutes gap junction communication and calcium wave transmission in glial cells. J Cell Biol 139:1785–1792.

Hebda PA, Collins MA, Tharp MD. 1993. Mast cell and myofibroblast in wound healing. Dermatol Clin 11:685–696.

Kischer CW, Bailey JF. 1972. The mast cell in hypertrophic scars. Tex Rep Biol Med 30:327–338.

Kischer CW, Bunce H III, Shetlah MR. 1978. Mast cell analyses in hypertrophic scars, hypertrophic scars treated with pressure and mature scars. J Invest Dermatol 70:355–357.

Kretz M, Euwens C. Hombach S, Eckardt D, Teubner B, Traub O, Willecke K, Ott T. 2003. Altered connexon expression and wound healing in the epidermis of connexon-deficient mice. J Cell Sci 116:3443–3452.

Kumar N, Gilula N. 1996. The gap junction communication channel. Cell 84:381–388.

Lampe PD, Nguyen BP, Gil S, Usui M, Olerud J, Takada Y, Carter WG. 1998. Cellular interaction of integrin $\alpha 3\beta 1$ with laminin 5 promotes gap junctional communication. J Cell Biol 143:1735–1747.

Matsushima R, Takahashi A, Nakaya Y, Maezawa H, Miki M, Nakamura Y, Ohgushi F, Yasuoka S. 2006. Human airway trypsin-like protease stimulates human bronchial fibroblast proliferation in a protease-activated receptor-2-dependent pathway. Am J Physiol Lung Cell Mol Physiol 290:L385–L395.

Moyer KE, Saggers GC, Ehrlich HP. 2004. Mast cells promote fibroblast populated collagen lattice contraction through gap junction intercellular communication. Wound Repair Regen 12:269–275.

Musil LS, Goodenough DA. 1991. Biochemical analysis of connexin43 intracellular transport, phosphorylation, and assembly into gap junctional plaques. J Cell Biol 115: 1357–1374.

Nishikori Y, Kakizoe E, Kobayashi Y, Shimoura K, Okunishi H, Dekio S. 1998. Skin mast cell promotion of matrix remodeling in burn wound healing in mice: Relevance of chymase. Arch Dermatol Res 290:553–560.

Oliani SM, Girol AP, Smith RL. 1995. Gap junctions between mast cells and fibroblasts in the developing avian eye. Acta Anat 154:267–271

Salomon D, Saurat JH, Meda P. 1988. Cell-to-cell communication within intact human skin. J Clin Invest 82:248–254.

Sasaki A, Mueller RV, Xi G, Sipe R, Buck D, Hollinger J. 2003. Mast cells: An unexpected finding in the modulation of cutaneous wound repair by charged beads. Plast Reconstr Surg 111:1446–1453.

Skold CM, Ohkuni Y, Liu XD, Numerof R, Rennard SI. 2001. Co-cultured human mast cells stimulate fibroblast-mediated contraction of collagen gels. Inflammation 25:47–51.

Smith CJ, Smith JC, Finn MC. 1987. The possible role of mast cells (allergy) in the production of keloid and hypertrophic scarring. J Burn Care Rehabil 8:126–131.

Trautmann A, Krohne G, Brocker EB, Klein CE. 1998. Human mast cells augment fibroblast proliferation by heterotypic cell-cell adhesion and action of IL-4. J Immunol 160:5053–5057.

Trautmann A, Toksoy A, Engelhardt E, Brocker EB, Gillitzer R. 2000. Mast cell involvement in normal human skin wound healing: Expression of monocyte chemoattractant protein-1 is correlated with recruitment of mast cells which synthesize interleukin-4 in vivo. J Pathol 190:100–106.

Wierecky J, Grabbe J, Wolff HH, Gibbs BF. 2000. Cytokine release from a human mast cell line HMC-1) in response to stimulation with anti-IgE and other secretagogues. Inflamm Res 49(Suppl 1):S7–S8.

Xu X, Rivkind A, Pappo O, Pikarsky A, Levi-Schaffer F. 2002. Role of mast cells and myofibroblasts in human peritoneal adhesion formation. Ann Surg 236:593–601.

Yamamoto T, Hartmann K, Eckes B, Krieg T. 2000. Mast cells enhance contraction of three-dimensional collagen lattices by fibroblasts by cell-cell interaction: Role of stem cell factor/c-kit. Immunology 99:435–439.