EXTENDED REPORTS

Expression of laminins and their integrin receptors in different conditions of synovial membrane and synovial membrane-like interface tissue

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Abstract

Objective—To demonstrate the expression of laminins (Lns) and their integrin (Int) receptors in different synovial samples and synovial membrane-like interface tissues from well fixed and aseptically loosened total hip replacement (THR), and the potential role of Ln-Int interaction in the production of collagenases and cytokines.

Methods—Immunohistochemical staining was done to detect the distribution of EHS Ln, Ln $\alpha 2$, $\alpha 3$, $\alpha 5$, $\beta 1$, $\beta 2$ chains and Int $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 6$, $\beta 1$, $\beta 4$ subunits in different samples. Double immunofluorescence labelling was used to find colocalisation of Int $\alpha 6$ subunit and collagenase-1/collagenase-3/TNF α /IL6.

Results—General Ln immunoreactivity was detected in all specimens. Ln $\alpha 5$, $\beta 1$ and $\beta 2$, but not $\alpha 2$ and $\alpha 3$ chains were seen in the synovial lining and the basement membrane of blood vessels with the intensity/extent of labelling in the following rank order: rheumatoid arthritis (RA) loosened prostheses, osteoarthritis, well fixed prostheses, traumatic knees. Among Int subunits, staining for $\beta 1$ was usually the strongest, followed by staining for Int $\alpha 6$, $\alpha 1$, $\alpha 3$, and $\alpha 2$ subunits, with the same rank order for overall expression of Lns. Int $\beta 4$ subunit was not detectable in most of the specimens. Double labelling focused on Int $\alpha 6$ subunit disclosed its frequent colocalisation with collagenases 1 and 3 and with tumour necrosis factor α and interleukin 6 in synovial lining.

Conclusion—Synovial lining contains Ln-10, Ln-11, and Int $\alpha 6\beta 1$ and $\alpha 1\beta 1$ receptors. In aseptic loosening of THR, interface tissue has a similar Ln subtype and Int receptor composition as RA synovium, which confirms its “lining-like” phenotype. Synovial lining does not contain Ln-5 ($\alpha 3\beta 3\gamma 2$) or Int $\alpha 6\beta 4$, which are components of epithelial hemidesmosomes. The expression of Lns and their Int receptors is upregulated in inflammation. The

close spatial relation between Ln and its Int receptors in synovial lining cells containing proteinases and cytokines suggests a potential role in joint destruction and prosthetic loosening.

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The formation of synovial membrane-like interface tissue between prostheses/cement and bone is a common pathological finding in aseptic loosening of total hip replacement (THR). The histological features of interface tissue are characteristic of a foreign body granuloma. It presents a similar bone lytic potential as rheumatoid arthritis (RA) synovitis tissue.¹ Most of the previous studies have been focused on the cell reactivity to wear debris and the effect of bioactive substances secreted by activated cells in periprosthetic osteolysis,² whereas little is known about the expression in the interface tissue and potential role of extracellular matrix (ECM) proteins in the process of prostheses loosening.

Laminin (Ln) was originally thought to be the third chain of type IV collagen while the resistance to collagenases indicated its non-collagenous nature. To date, five α , three β and two γ chains assembled into 11 different Lns have been clearly identified.³ Ln network is part of basal lamina and intercellular substance in the synovial lining. The abilities of Lns to stimulate cell adhesion and migration, as well as to influence gene expression, underlie their critical importance in development, differentiation and tissue homeostasis.⁴

The cell binding to Lns occurs via a variety of cell surface receptors. Integrins are the main way that cells both bind and respond to the ECM. Int $\alpha 6\beta 1$ has been demonstrated to be a major receptor of Lns while the well known collagen receptors, Int $\alpha 1\beta 1$ and $\alpha 2\beta 1$ can also bind to some Lns.^{5–7} The interaction of Lns and their Int receptors can, by influencing the organisation of cytoskeleton, activate intracellular signalling cascades and thus modulate the expression of proteases and cytokines in different tissues and cells.^{8–11}

Table 1 Antibodies used

Clone	Target	Source	Dilution	Reference
Polyclonal	EHS Ln	Rb	1:100	12
5H2	Ln- α 2	M	1:800	13
BM-2	Ln- α 3	M	1:400	14
4C7	Ln- α 5	M	1:800	15
LnB1	Ln- β 1	M	1:2000	16
C4	Ln- β 2	M	1:100	17
TS2/7	Int- α 1	M	1:30	18
CLB10G11	Int- α 2	M	1:600	19
J143	Int- α 3	M	1:300	20
GoH3	Int- α 6	R	1:600	21
102DF5	Int- β 1	M	1:300	22
AA3	Int- β 4	M	1:600	23

Ln-: different Ln chains; Int-: different integrin subunits, Rb: rabbit; M: mouse; R: rat.

This study was intended to show the distribution of Lns and Int receptors in different conditions of synovial membranes and synovial membrane-like interface tissue from well fixed and aseptically loosened hip prostheses and the eventual effect of Ln-Int interaction on the production of local bioactive substances

that contribute to joint destruction and periprosthetic osteolysis.

Methods

PATIENTS AND SAMPLES

Ten synovial samples were obtained from patients with osteoarthritis (OA) undergoing primary THR (seven women and three men, mean age 71.1 years, range 41–84). Five synovial samples were obtained from patients with RA undergoing primary THR (two men and three women, mean age 41.6 years, range 40–72). Five samples were collected from the interfaces between prostheses/cement and bone from OA patients undergoing revision THR because of aseptic loosening (three men and two women, mean age 73.6 years, range 61–86). The average time from primary operation to revision was 9.2 years (range 5–17). Five samples were collected from OA patients with well fixed implants from bone-cement interface; indication for the operation was a

Figure 1 EHS Ln immunoreactivity in synovial membrane and interface tissue. The staining was comparatively strong in the lining layers (arrows), sublining area and basement membrane of blood vessels. Weak staining was occasionally found in deep stroma in the pericellular spaces. The staining in RA synovial membrane was usually the strongest (A), followed by interface tissue from aseptic loosening of THR (B), OA synovial membrane (C), the samples from well fixed THR (D) and the synovial samples from traumatic knees (E). For staining control the specific primary rabbit antiserum to human EHS Ln was replaced with rabbit IgG (F).

Figure 2 EHS Ln, Ln chains and Int subunits in semi-serial sections of a synovial sample from OA. Panel A: EHS Ln, B: Ln $\alpha 2$ chain, C: Ln $\alpha 3$ chain, D: Ln $\alpha 5$ chain, E: Ln $\beta 1$ chain, F: Ln $\beta 2$ chain. Panel G: Int $\alpha 1$ subunit, H: $\alpha 2$ subunit, I: $\alpha 3$ subunit, J: $\alpha 6$ subunit, K: $\beta 1$ subunit, L: $\beta 4$ subunit.

mechanical failure of the polyethylene cup (three women and two men, mean age 65.8 years, range 59–70). None of them had clinical or radiological signs of aseptic loosening. The mean time from the primary to the second operation was 5.6 years (1–10). In addition, “non-inflammatory” synovial membrane samples were obtained from five patients undergoing arthroscopy because of knee injuries (two men and three women, mean age 40.6 years, range 22–53). The final diagnosis turned out to be injury of meniscus (three cases) and anterior cruciate ligament tear (two cases). All samples were embedded in OCT compound (Lab-Tek Products, Miles Laboratories, Elkhart, IN) and frozen in isopentane precooled by dry ice. The frozen samples were stored at -70°C until use.

IMMUNOHISTOCHEMISTRY

Six μm thick cryostat sections were fixed in cold acetone for 15 minutes at -20°C . Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in absolute methanol at 22°C for 30 minutes. The sections were then treated according to the following procedures: (1) Incubation with the following normal sera (Vector Laboratories, Burlingame, CA; dilution 1:50 in TRIS buffered saline (TBS)

containing 0.1% bovine serum albumin (BSA)) at 22°C for 20 minutes: (a) goat serum for polyclonal Ln antibody; (b) rabbit serum for Int $\alpha 6$ subunit; (c) horse serum for the mouse MAbs. (2) Incubation with primary antibodies (diluted in TBS containing 0.1% BSA, see table 1) overnight at 4°C . (3) Incubation with following biotinylated antibodies (Vector Laboratories, diluted 1:100 in TBS containing 0.1% BSA) at 22°C for 30 minutes respectively: (a) goat antirabbit IgG; (b) horse antimouse IgG; (c) rabbit antirat IgG. (4) Incubation with avidin-biotin-peroxidase complex (ABC, Vector Laboratories, diluted 1:100 in TBS containing 0.1% BSA) for 30 minutes at 22°C . (5) Incubation with a combination of 3,3-diaminobenzidine tetrahydrochloride (DAB, Sigma, St Louis, MO; 35 mg dissolved in 150 ml TBS) and 0.006% H_2O_2 at 22°C for five minutes. Between two steps, the sections were washed for 3×5 minutes in TBS. The sections were dehydrated in a graded ethanol series, cleared in xylene and mounted in Diatex. Instead of primary antibodies, normal rabbit IgG, rat and mouse IgG, with irrelevant specificities were used at the same concentration as negative staining controls.

Table 2 Staining scores of Ln chains and Int subunits

Tissues	Ln- α 2	Ln- α 3	Ln- α 5	Ln- β 1	Ln- β 2	Int- α 1	Int- α 2	Int- α 3	Int- α 6	Int- β 1	Int- β 4
RA-1	±	–	+++	+++	+++	++	–	±	++	+++	±
RA-2	–	–	+++	+++	+++	+++	–	±	+++	+++	–
RA-3	–	±	+++	+++	+++	++	±	–	+++	+++	–
RA-4	±	±	+++	++	+++	++	–	–	++	+++	–
RA-5	–	–	+++	+++	+++	++	–	±	+++	+++	–
IT-1	±	–	++	++	++	+	±	–	++	+++	–
IT-2	–	–	+++	++	++	+	–	–	++	++	–
IT-3	–	–	++	++	+++	++	–	±	+++	++	–
IT-4	–	–	++	++	+++	++	–	±	++	+++	–
IT-5	±	–	+++	++	++	+	–	–	++	+++	±
OA-1	–	–	+	+	+	+	–	–	+	++	–
OA-2	–	–	+	+	++	+	–	–	+	++	–
OA-3	–	–	++	+	++	+	–	–	+	++	–
OA-4	±	–	++	++	+	+	–	–	++	++	±
OA-5	–	–	+	+	++	+	–	–	+	++	–
OA-6	–	–	++	+	++	±	–	–	+	++	–
OA-7	–	–	+	+	+	±	–	–	++	++	–
OA-8	–	±	+	+	+	+	–	–	+	++	–
OA-9	–	–	+	++	++	+	–	±	++	++	–
OA-10	–	–	+	+	++	±	–	–	+	++	–
WF-1	–	–	±	±	±	–	–	–	+	++	–
WF-2	–	–	+	±	+	±	–	–	+	+	–
WF-3	–	–	+	+	+	±	–	–	+	+	–
WF-4	–	–	+	±	±	±	–	–	±	+	–
WF-5	±	±	+	+	+	±	–	–	+	++	–
KA-1	–	±	+	±	±	±	–	–	±	±	–
KA-2	–	–	±	±	±	±	–	–	±	±	–
KA-3	–	–	+	±	±	–	–	–	±	±	–
KA-4	–	–	±	+	+	–	–	–	+	+	–
KA-5	–	–	±	±	±	±	–	–	±	+	–

RA: synovial samples from patients with RA; IT: synovial membrane-like interface tissue from patients with aseptic loosening of THR; OA: synovial samples from patients with OA; WF: the samples from well fixed bone-cement/prosthesis interface; KA: synovial samples from patients with traumatic knees undergoing arthroscopy.

For light microscopical analysis, the stained specimens were evaluated for the overall extent and intensity of staining into no staining (–), weak staining (±), moderate staining (+), strong staining (++), and very strong staining (+++).

DOUBLE IMMUNOFLUORESCENCE LABELLING FOR INT α 6 SUBUNIT AND MMP-1/MMP-13/TNFA/IL6
Cryostat sections (6 μ m) were cut from five samples of OA synovial membranes and five samples of interface tissues. After fixation in cold acetone at –20°C for 20 minutes, the sections were washed with PBS and incubated with the following antibodies: (1) rat antihuman IgG against Int α 6 subunit (diluted 1:250 in PBS containing 1.25% BSA) for 60 minutes at 22°C. (2) FITC conjugated donkey antirat IgG (diluted 1:100 in PBS containing 12.5% BSA, Jackson ImmunoResearch Laboratories, West Grove, PA) at 22°C for 45 minutes in the dark. (3) Polyclonal rabbit antihuman collagenase-1 (MMP-1) IgG (diluted 1:1000 in PBS containing 1.25% BSA, Chemicon International Inc, Temecula, CA) at 22°C for 60 minutes. (4) TRITC conjugated goat anti-rabbit IgG (diluted 1:100 in PBS containing 12.5% BSA, Jackson ImmunoResearch Laboratories) at 22°C for 45 minutes in the dark.

Human IgG (diluted 0.8/1 in PBS, Sigma) was added to the conjugated second antibody solutions to reduce non-specific reaction. The sections were washed with PBS for 3 $\times$ 10 minutes between two steps. The slides were air dried and mounted with Vectashield (Vector Laboratories) and kept in the dark at 4°C. For negative control, rat IgG₁ and rabbit IgG were used instead of the first and second primary antibodies respectively.

Similar protocols were used for Int α 6 subunit and collagenase-3 (MMP-13)/tumour

necrosis factor α (TNF α)/interleukin 6 (IL6) double staining for this purpose, the following polyclonal rabbit antisera were used: antiserum to MMP-13 (1:300;²⁴), to TNF α (1:200; Monosan, Udne, the Netherlands), and to IL6 (1:200; Genzyme, Cambridge, MA).

The specimens were viewed through a Leitz Aristoplan fluorescence microscope equipped with epi-illumination and specific filters: filter block I3 for FITC (excitation filters BP 450–490, dichromatic mirrors RKP 510, suppression filters 520), and filter block N2.1 for TRITC (excitation filters BP 515–560, dichromatic mirror RKP 580, suppression filter LP 580). The photographs were taken on Kodak T-max film (Rochester, NY) using an automatic Leitz Vario-Orthomat microscope camera.

Results

GENERAL LN IMMUNOREACTIVITY

All samples showed immunoreactivity for EHS Ln. Relatively strong staining was found in the lining layers and sublining area of synovial membrane, lining-like layers of interface tissue and the basal laminae of the blood vessels. Occasionally, immunoreactivity could be found around cells in deep stroma. The strongest reaction appeared in RA samples, followed by interface tissue from aseptic loosening of THR and OA samples, whereas staining of well fixed cement/prosthesis-bone interface and traumatic knee synovium was weak and confined to pericellular areas in lining layer (figs 1, 2).

LOCALISATION OF DIFFERENT LN CHAINS

Ln α 5, β 1 and β 2 chain immunoreactivities were found in all of the samples. The distribution of α 5 and β 1 chains was similar and relatively restricted. The staining for these two

Figure 3 Int $\alpha 6$ subunit staining was intense around the cells in the lining layer and in vascular endothelial cells. Occasionally, weak cellular staining was detected in sublining and deep stroma. Staining was the strongest in RA samples (A), followed by interface tissue from aseptic loosening of THR (B), OA synovial membrane (C), interface tissue from well fixed prostheses (D) and synovial membrane from knee injuries (E). For staining control the primary mouse antihuman Int $\alpha 6$ IgG₁ was replaced with monoclonal IgG₁ with an irrelevant specificity (F).

chains was strikingly strong in the basal laminae of blood vessels although staining of lining and sublining region was also detectable. In contrast, the staining of Ln $\beta 2$ chain was more extensive, but in the basal laminae of blood vessels not as strong as that of the Ln $\alpha 5$ and $\beta 1$ chains. Pericellular Ln $\beta 2$ chain reactivity often appeared in deep stroma. In most of the samples, Ln $\alpha 2$ and $\alpha 3$ chains were not detectable (table 2, fig 2). The distribution patterns of Ln $\alpha 5$, $\beta 1$ and $\beta 2$ chains were similar, but the intensity of staining varied greatly between different sample categories.

DISTRIBUTION OF DIFFERENT INT SUBUNITS

Among all the Int subunits stained, the staining of Int $\beta 1$ subunit was usually the strongest, followed by staining of Int $\alpha 6$ and $\alpha 1$ subunits. The distribution patterns for these Int subunits were similar, for example, the strong staining of $\alpha 6$ subunit was always detected around the cells

in lining layers of synovial membrane, the lining-like layers of interface tissue and vascular endothelial cells (fig 3). The immunoreactivity of Int $\alpha 3$ subunit was very weak and occasionally found in vascular endothelial cells in the stroma. In most cases, staining for Int $\alpha 2$ and $\beta 4$ subunits was not detectable (table 2, fig 2). Int $\alpha 6$, $\alpha 1$ and $\beta 1$ staining was the strongest in RA synovium, followed in the rank order by interface tissues from loosened prostheses, OA synovium, the samples from well fixed interface, and synovial samples from traumatic knees (fig 3).

DOUBLE IMMUNOFLOUORESCENCE STAINING FOR INT $\alpha 6$ SUBUNIT AND MMP-1, MMP-13, TNF α OR IL6

In the lining layer of OA synovium (fig 4), there were some Int $\alpha 6$ subunit/MMP-1 (A, B), Int $\alpha 6$ subunit/MMP-13 (C, D), Int $\alpha 6$ subunit/TNF α (E, F) and Int $\alpha 6$ subunit/IL6 (G, H) double positive cells. Colocalisation of Int $\alpha 6$

Figure 4 Double immunofluorescence labelling for Int $\alpha 6$ subunit and MMP-1, MMP-13, TNF- α or IL6 in OA. Some of the double positive cells have been marked with arrows. Panel A and B: $\alpha 6$ subunit and MMP-1 double labelling, A: $\alpha 6$ positive cells, B: MMP-1 positive cells; Panel C and D: $\alpha 6$ subunit and MMP-13 double labelling, C: $\alpha 6$ positive cells, D: MMP-13 positive cells; Panel E and F: $\alpha 6$ subunit and TNF α double labelling, E: $\alpha 6$ positive cells, F: TNF α positive cells; Panel G and H: $\alpha 6$ subunit and IL6 double labelling, G: $\alpha 6$ positive cells, H: IL6 positive cells.

subunit and collagenases/cytokines was rarely found in deep stroma. In the lining-like layers of interface tissue from aseptic loosening of THR (fig 5), there were many Int $\alpha 6$ subunit/MMP-1 (A, B), Int $\alpha 6$ subunit/MMP-13 (C, D), Int $\alpha 6$ subunit/TNF α (E, F) and Int $\alpha 6$ subunit/IL6 (G, H) double positive cells. Colocalisation of $\alpha 6$ subunit with collagenases and cytokines was occasionally found in the endothelial and macrophage-like cell of the stroma.

Discussion

The main molecular components of BM are type IV collagen, nidogen/entactin and Lns. These molecules are present in the ECM surrounding the synovial lining cells, but in contrast with their organisation in basement membrane (BM), they do not form a typical trilaminar structure separating the lining cells and stroma of synovium. This makes synovial lining a unique cell-matrix composite structure in human body.²⁵

The multidomain nature of Ln is ideally suited to mediate the interactions with a variety of BM molecules. Their primary role is in cell-

matrix attachment, but many additional biological activities, including promotion of cell growth and migration, wound repair and graft survival, have been demonstrated. The diverse functions of Lns in different tissues may be attributable to the existence of a variety of Ln molecule isoforms. These isoforms can differ from each other by one, two or all three constituent chains.³

Identification of Ln $\alpha 1$ chain was earlier based on the use of MAb 4C7 raised against human placental Ln. More recently, it has been noticed that it actually reacts with a 380 kDa Ln $\alpha 5$ chain. The idea of widespread expression of Ln $\alpha 1$ chain and Ln-1 came from studies done with MAb 4C7. In fact, the distribution of Ln $\alpha 1$ chain is very restricted, it is only found in adult mouse, rat and human kidney. In contrast, Ln $\alpha 5$ chain is more widely distributed.²⁶⁻²⁸ All the results from the studies with MAb 4C7 should, therefore, be reinterpreted as the expression of Ln $\alpha 5$ chain.²⁹ Ln $\alpha 5$ chain contains the domain VI, which mediates the intermolecular assembly of Ln heterotrimers in other Ln types.³⁻³⁰ Our study showed intercellular spaces of the intima/synovial lining and the basal laminae of blood vessels contain Ln $\alpha 5$ chain together with Ln $\beta 1$ chain. Although they were detected in the nearly normal synovial membrane from traumatic knees, the staining was much stronger and more widespread in the synovial samples from RA and interface tissue from loosened prostheses. These results indicate that synovial lining contains, not Ln-1 ($\alpha 1\beta 1\gamma 1$), but Ln-10 ($\alpha 5\beta 1\gamma 1$), and that the production of Ln-10 is upregulated in inflammation.

Interestingly, Ln $\beta 2$ chain was found in synovial samples and interface tissue. It was originally identified as a component of the BM of the neuromuscular junction¹⁷ and has subsequently been found in the BMs of the kidney glomerulus, peripheral nerve, vascular smooth muscle, lung alveoli, seminiferous epithelium, amnion, eye, sarcolemma and several parts of the central nervous system.^{16 31-38} This is the first report about the coexistence of Ln $\alpha 5$ and $\beta 2$ chains in synovial membrane and interface tissue, indicating that Ln-11 ($\alpha 5\beta 2\gamma 1$), in addition to Ln-10, exist in these tissues, and that inflammation also increases local expression of Ln-11.

Lack of BM in synovium and interface tissue facilitates the deposition of Ln. Locally produced bioactive substances, such as cytokines, growth factors, and nitric oxide may, as paracrine effectors, increase Ln synthesis.³⁹⁻⁴² The production of these substances are increased in arthritis and aseptic loosening of THR,⁴³⁻⁴⁶ which may contribute to the upregulated Ln expression in RA synovium and interface tissue from aseptic loosening of THR.

Integrins confer specificity and signalling functions through relatively low affinity interactions while non-Int proteins stabilise and strengthen Int initiated interactions.^{6 7} Although it is generally established that Int receptors of Lns are not necessarily designed to be specific because of the complexity of Ln-receptor interaction, Int $\alpha 6\beta 1$ has been

Figure 5 Double immunofluorescence labelling for Int $\alpha 6$ subunit and MMP-1, MMP-13, TNF α or IL6 in synovial membrane-like interface tissues from a patient with aseptic loosening of THR. Panel A and B: a6 subunit and MMP-1 double labelling, A: a6 positive cells, B: MMP-1 positive cells; Panel C and D: a6 subunit and MMP-13 double labelling, C: a6 positive cells, D: MMP-13 positive cells; Panel E and F: a6 subunit and TNF α double labelling, E: a6 positive cells, F: TNF α positive cells; Panel G and H: a6 subunit and IL6 double labelling, G: a6 positive cells, H: IL6 positive cells.

identified as the main receptor for many Lns.³ In this study, we show that Int $\alpha 6$ subunit appears in all synovial samples, especially in RA and aseptic loosening of THR. Because the same samples also express Int $\beta 1$ subunit, we conclude that Int $\alpha 6\beta 1$ complex is the main receptor for Lns in these tissues. In addition, Int $\alpha 1\beta 1$ may also play a part in Ln-Int interaction in synovial lining and some samples contained also Int $\alpha 3\beta 1$. In contrast, Int $\alpha 2\beta 1$ and $\alpha 6\beta 4$ were not found. Lack of Int $\alpha 6\beta 4$, the Int component of the epithelial hemidesmosomes, was well in accordance with the non-epithelial nature of the synovial lining. Recruitment of Ints is Ln isoform specific. A series of proinflammatory cytokines can increase Int $\alpha 6$ synthesis, and increased amount of these cytokines have been found in RA and aseptic loosening of THR, which may explain the upregulated expression of Int $\alpha 6$ in these conditions.^{40 43 47-50}

Increased level of ECM proteins in inflamed synovium suggests that intracellular signals generated through Int receptors might control the process of joint destruction. Proliferation of synovial fibroblasts from RA requires a signal provided by ECM in addition to a signal

provided by a mitogenic cytokine. ECM engagement without attendant cytoskeletal formation leads to high level of collagenase expression associated with a change in cell shape of synovial fibroblasts.⁵¹ The recombinant fragments, proteolytic fragments and synthetic peptides of mouse Ln-1 have been shown to stimulate plasminogen activation and to upregulate collagenase activity without modulating the expression of tissue inhibitors of metalloproteinases (TIMP). In this study, we have shown that there were many Int $\alpha 6$ subunit and collagenase-1 (MMP-1) or collagenase-3 (MMP-13) double positive cells in synovial lining, implicating that intact Lns, by interacting with cell surface Int receptors might also modulate the production of collagenases. Although colocalisation only implies associative and not necessarily causative relation, this might in part explain the increased expression of collagenases in interface tissues from aseptic loosening of THR.^{52 53}

Ln-Int interaction can regulate the production of some cytokines.^{9 10} In this study, we have detected some Int $\alpha 6$ subunit and TNF α /IL6 double positive cells in lining layers of different tissues. The number of such double positive cells was higher in the interface tissues than in OA synovium. Our results suggest a role for Ln-Int interaction in the production of these cytokines. Both cytokines have been shown to be involved in the joint destruction and aseptic loosening of THR.^{43 48 54 55}

In conclusion, synovial lining contains, not Ln-1, but Ln-10 and Ln-11 and $\alpha 6\beta 1$ and $\alpha 1\beta 1$ type Ln receptors. Interface tissue from aseptic loosening of THR has a similar Ln subtype and Int receptor composition, which confirms its "lining-like" phenotype.⁵⁶ The fact that snovial lining does not contain Ln-5 ($\alpha 3\beta 3\gamma 2$) and Int $\alpha 6\beta 4$ differentiates it from epithelia. The expressions of Lns and their Int receptors are upregulated in the conditions such as RA and aseptic loosening of THR. The close spatial relation between Lns and their Int receptors in synovial lining cells containing proteinases and cytokines suggests an important potential in the regulation of the phenotype and function of these cells. Such interactions might play a part in the pathogenesis of arthritis and prosthetic loosening.

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- Goldring SR, Schiller AI, Roelke M, Rourke CM, O'Neill DA, Harris WH. The synovial-like membrane at the bone-cement interface in loose total hip replacements and its proposed role in bone lysis. *J Bone Joint Surg* 1983;65A:575-84.
- Lassus J, Salo J, Jiranek WA, Santavirta S, Nevalainen J, Matucci-Cerinic M, *et al.* Macrophage activation results in bone resorption. *Clin Orthop* 1998;352:7-15.
- Engvall E, Wewer UM. Domains of laminin. *J Cell Biochem* 1996;61:493-501.
- Timpl R, Brown J. Supramolecular assembly of basement membranes. *Bioassay* 1996;18:123-32.
- Sonnenberg A, Linders CJ, Modderman PW, Damsky CH, Aumailley M, Timpl R. Integrin recognition of different cell-binding fragments of laminin (P1, E3, E8) and evidence that $\alpha 6\beta 1$ but not $\alpha 6\beta 4$ functions as major receptor for fragment E8. *J Cell Biol* 1990;110:2145-55.
- Mercurio AM. Laminin receptors: achieving specificity through cooperation. *Trend Cell Biol* 1995;5:419-23.
- Eklöf P. Receptors for laminins during epithelial morphogenesis. *Curr Opin Cell Biol* 1996;8:700-6.

- 8 Stack S, Gray RD, Pizzo SV. Modulation of plasminogen activation and type IV collagenase activity by a synthetic peptide derived from the laminin A chain. *Biochem* 1991; 30:2073-7.
- 9 Miyake S, Yagita H, Maruyama T, Hashimoto H, Miyasaka K, Okumura K. Beta 1 integrin-mediated interaction with extracellular matrix proteins regulates cytokine gene expression in synovial fluid cells of rheumatoid arthritis patients. *J Exp Med* 1993;177:863-8.
- 10 Derevianko A, D'Amico R, Simms H. Polymorphonuclear leukocyte (PMN)-derived inflammatory cytokines-regulation by oxygen tension and extracellular matrix. *Clin Exp Immunol* 1996;106:560-7.
- 11 Khan KMF, Falcone DJ. Role of laminin in matrix induction of macrophage urokinase-type plasminogen activation and 92-kDa metalloproteinase expression. *J Biol Chem* 1997;272:8270-5.
- 12 Liesi P, Dahl D, Vaehri A. Laminin is produced by early rat astrocytes in primary culture. *J Cell Biol* 1983;96:920-4.
- 13 Leivo I, Engvall E. Merosin, a protein specific for basement membranes of Schwann cells, striated muscle, and trophoblast, is expressed late in nerve and muscle development. *Proc Natl Acad Sci USA* 1988;85:1544-8.
- 14 Rousselle P, Lunstrum GP, Keene DR, Burgeson RE. Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. *J Cell Biol* 1991;114:567-76.
- 15 Engvall E, Davis GE, Dickerson K, Ruoslahti E, Varon S, Manthorpe M. Mapping of domains in human laminin using monoclonal antibodies. *J Cell Biol* 1986;103:2457-65.
- 16 Virtanen I, Lohi J, Tani T, Korhonen M, Burgeson RE, Lehto VP, et al. Distinct changes in the laminin composition of basement membranes in human seminiferous tubules during development and degeneration. *Am J Pathol* 1997;150:1421-31.
- 17 Hunter DD, Shah V, Merlie JP, Sanes JR. A laminin-like adhesion protein concentrated in the synaptic cleft of the neuromuscular junction. *Nature* 1989;338:229-34.
- 18 Helmer ME, Sanchez-Madrid F, Flotte TJ, Krensky AM, Burakoff SJ, Bhan AK, et al. Glycoproteins of 210,000 and 130,000 M.W. on activated T cells. *J Immunol* 1984;132:3011-18.
- 19 Giltay JC, Brinkman HJ, Modderman PW, von dem Borne AE, van Mourik JA. Human vascular endothelial cells express a membrane protein complex immunochemically indistinguishable from the platelet VLA-2 (glycoprotein Ia-IIa) complex. *Blood* 1989;73:1235-41.
- 20 Fradet Y, Cordon-Cardo C, Thomson T, Daly ME, Whitmore WF Jr, Lloyd KO, et al. Cell surface antigens of human bladder cancer defined by mouse monoclonal antibodies. *Proc Natl Acad Sci USA* 1984;81:224-8.
- 21 Sonnenberg A, Janssen H, Hogervorst F, Calafat J, Hilgers J. A complex of platelet glycoprotein Ic and IIa identified by a rat monoclonal antibody. *J Biol Chem* 1987;262:10376-83.
- 22 Ylännä J, Virtanen I. The Mr 140,000 fibronectin receptor complex in normal and virus-transformed human fibroblasts and fibrosarcoma cells. *Int J Cancer* 1989;43:1126-36.
- 23 Tamura RN, Rozzo C, Starr L, Chambers J, Reichardt LF, Cooper HM, et al. Epithelial integrin $\alpha 6 \beta 4$ complete primary structure of $\alpha 6$ and variant forms of $\beta 4$. *J Cell Biol* 1991;111:1539-604.
- 24 Lindy O, Kontinen YT, Sorsa T, Ding Y, Santavirta S, Ceponis A, et al. Matrix metalloproteinase 13 (collagenase-3) in human rheumatoid synovium. *Arthritis Rheum* 1997;40:1391-9.
- 25 Nikkari L, Aho H, Yli-Jama T, Larjava H, Jalkanen M, Heino J. Expression of integrin family of cell adhesion receptors in rheumatoid synovium. Alpha 6 integrin subunit in normal and hyperplastic synovial lining cell layer. *Am J Pathol* 1993;142:1019-27.
- 26 Salmivirta K, Sorokin LM, Ekblom P. Differential expression of laminin α chains during murine tooth development. *Dev Dyn* 1997;210:206-15.
- 27 Sorokin LM, Pausch F, Frieser M, Kröger S, Ohage E, Deutzman R. Developmental regulation of laminin $\alpha 5$ suggests a role in epithelial and endothelial maturation. *Dev Biol* 1997;189:285-300.
- 28 Sorokin LM, Pausch F, Durbeek J, Ekblom P. Differential expression of five laminin α (1-5) chains in developing and adult mouse kidney. *Dev Dyn* 1997;210:446-62.
- 29 Tiger CF, Champlaud MF, Pedrosa-Domellof F, Thornell LE, Ekblom P, Gullberg D. Presence of laminin $\alpha 5$ chain and lack of laminin $\alpha 1$ chain during human muscle development and in muscular dystrophies. *J Biol Chem* 1997;272:28590-5.
- 30 Miner JH, Patton BL, Lentz SI, Gilbert DJ, Snider WD, Jenkins NA, et al. The laminin α chains: expression, developmental transitions, and chromosomal locations of $\alpha 1$ -5, identification of heterotrimeric laminin 8-11, and cloning of a novel $\alpha 3$ isoform. *J Cell Biol* 1997;137:685-701.
- 31 Sanes JR, Engvall E, Butkowski R, Hunter DD. Molecular heterogeneity of basal laminae: isoforms of laminin and collagen IV at the neuromuscular junction and elsewhere. *J Cell Biol* 1990;111:1685-99.
- 32 Wewer UM, Gerecke DR, Durkin ME, Kurtz KS, Mattei MG, Champlaud MF, et al. Human $\beta 2$ chain of laminin (formerly S chain): cDNA cloning, chromosomal localization, and expression in carcinomas. *Genomics* 1995;24:243-52.
- 33 Champlaud MF, Lunstrum GP, Rousselle P, Nishiyama T, Keene DR, Burgeson RE. Human amnion contains a novel limonene variant, limonene 7, which like limonene 6, covalently associates with limonene 5 to promote stable epithelial-stromal attachment. *J Cell Biol* 1996;132:1189-98.
- 34 Virtanen I, Laitinen L, Korhonen M. Differential expression of laminin polypeptides in developing and adult human kidney. *J Histochem Cytochem* 1995;43:621-8.
- 35 Virtanen I, Laitinen A, Tani T, Pääkkö M, Laitinen LA, Burgeson RE, et al. Different expression of laminins and their receptors in developing and adult human lung. *Am J Respir Cell Mol Biol* 1996;15:184-96.
- 36 Hunter DD, Murphy MD, Olsson CV, Brunken WJ. S-laminin expression in adult and developing retinae. *Neuron* 1992;8:399-413.
- 37 Wewer UM, Thornell LE, Loechel F, Zhang X, Durkin ME, Amano S, et al. Extrasynaptic location of laminin $\beta 2$ chain in developing and adult human skeletal muscle. *Am J Pathol* 1997;151:621-31.
- 38 Jaakkola S, Savunen O, Halme T, Uitto J, Peltonen J. Basement membranes during development of human nerve: Schwann cells and perineurial cell display marked changes in their expression profiles for laminin subunits and $\beta 1$ and $\beta 4$ integrin. *J Neurocytol* 1993;22:215-30.
- 39 Moutabarrik A, Nakanishi I, Zaid D, Namiki M, Kawaguchi N, Onishi S, et al. Interleukin-1 β activation of cultured glomerular epithelial cells. *Exp Nephrol* 1994;2:196-204.
- 40 Kumar NM, Sigurdson SL, Sheppard D, Lwebuga-Mukasa JS. Different modulation of integrin receptors and extracellular matrix laminin by transforming growth factor- $\beta 1$ in rat alveolar epithelial cells. *Exp Cell Res* 1995;221:385-94.
- 41 Trachtman H, Futterweit S, Singhal P. Nitric oxide modulates the synthesis of extracellular matrix proteins in cultured rat mesangial cells. *Biochem Biophys Res Commun* 1995;207:120-5.
- 42 Vasaturo F, Modesti A, Scarp S. Interferon- γ modifies fibronectin and laminin synthesis in human neuroblastoma cell lines. *Int J Oncol* 1998;12:895-8.
- 43 Kontinen YT, Kurvinen H, Tagaki M, Michelsson JE, Eklund KK, Nordsletten L, et al. Interleukin-1 vs collagenase around total hip prostheses. *Clin Exp Rheumatol* 1996;14:255-62.
- 44 Kontinen TY, Warris V, Xu JW, Jiranek WA, Sorsa T, Virtanen I, et al. Transforming growth factor- $\beta 1$ and 2 in the synovial-like interface membrane between implant and bone in loosening of total hip arthroplasty. *J Rheumatol* 1997;24:694-701.
- 45 Hukkanen M, Corbett SA, Platts LA, Kontinen YT, Santavirta S, Hughs SP, et al. Nitric oxide in the local host reaction to total hip replacement. *Clin Orthop* 1998;352:53-65.
- 46 Goodman SB, Hui P, Song Y, Schurman D, Maloney W, Woollen S, et al. Cellular profile and cytokine production at prosthetic interface. *J Bone Joint Surg* 1998;80B:5631-9.
- 47 Felch ME, Willis RA, Penney DP, Keng PC, Phipps RP. Expression of $\alpha 6 \beta 1$, the laminin receptors, on subset of normal murine lung fibroblasts and its upregulation by the inflammatory cytokines IFN- γ and TNF- α . *Reg Immunol* 1992;4:363-70.
- 48 Xu JW, Kontinen YT, Lassus J, Natah S, Ceponis A, Solivieva S, et al. Tumor necrosis factor- α (TNF- α) in loosening of total hip replacement (THR). *Clin Exp Rheumatol* 1996;14:643-8.
- 49 Dogic D, Rousselle P, Aumailley M. Cell adhesion to laminin 1 or 5 induces isoform-specific clustering of integrins and other focal adhesion components. *J Cell Sci* 1998;111:793-802.
- 50 Santala P, Heino J. Regulation of integrin-type cell adhesion receptors by cytokines. *J Biol Chem* 1991;266:23505-9.
- 51 Sarkissian M, Lafyatis R. Integrin engagement regulates proliferation and collagenase expression of rheumatoid synovial fibroblasts. *J Immunol* 1999;162:1772-9.
- 52 Takagi M, Santavirta S, Ida H, Ishii M, Akimoto K, Saotome K, et al. The membrane-type-matrix metalloproteinases/matrix metalloproteinases-2/tissue inhibitor of metalloproteinases-2 system in periprosthetic connective-tissue remodeling in loose total hip prostheses. *Lab Invest* 1998;78:735-42.
- 53 Imai S, Kontinen YT, Juppunen M, Lindy O, Ceponis A, Kempainen P, et al. High levels of expression of collagenase-3 (MMP-13) in pathological conditions associated with a foreign-body reaction. *J Bone Joint Surg* 1998;80B:701-10.
- 54 Deleuran BW, Chu CQ, Field M, Brennan FM, Mitchell T, Feldmann M, et al. Localization of tumor necrosis factor receptors in the synovial tissue and cartilage-pannus junction in patients with rheumatoid arthritis. *Arthritis Rheum* 1992;35:1170-8.
- 55 Yanni G, Whelan A, Feighery C, Bresnihan B. Synovial tissue macrophages and joint erosion in rheumatoid arthritis. *Ann Rheum Dis* 1994;53:39-44.
- 56 Pollock LE, Lalor P, Revell PA. Type IV collagen and laminin in the synovial intimal layer. *Rheumatol Int* 1990;9:277-80.