Type I Collagen is a Non-Adhesive Extracellular Matrix for Macrophages

Yoh-ichi Koyama¹,², Keiko Norose-Toyoda¹, Seishiro Hirano³, Miya Kobayashi¹, Tetsuya Ebihara¹, Iori Someki¹, Hitomi Fujisaki¹ and Shinkichi Irie¹,²

Nippi Research Institute of Biomatrix¹, Nippi Inc., Tokyo; Department of Biomatrix Engineering¹; Japan Institute of Leather Research, Tokyo; Regional Environment Division³, National Institute for Environmental Studies, Tsukuba; and Department of Anatomy¹, Nagoya University School of Medicine, Nagoya, Japan

Received December 17, 1999

Summary. Macrophages adhere to a variety of substrata including plastic, glass or an extracellular matrix either in a highly specific manner or through less specific mechanisms. We investigated the effect of type I collagen, the most abundant protein in animal tissues, on the adhesion of macrophages derived from a human monoblastic cell line U937. Macrophages were observed to adhere very weakly to type I collagen and aggregate, whereas they adhered firmly and spread on plastic, bovine serum albumin or fibronectin. On the adhesive substratum, the lower surface of the macrophages was flat and closely apposed to the substratum. In contrast, macrophages adhered on type I collagen at the tip of cell processes. The adhesion of macrophages to plastic, bovine serum albumin or fibronectin was associated with the induction of tyrosine phosphorylation of a variety of proteins including a major protein band at 66 kDa. In contrast, the induction of tyrosine phosphorylation was markedly reduced when the macrophages were cultured on type I collagen. Two members of the src family, Lyn and Hck, were tyrosine phosphorylated in firmly adhered macrophages but not in macrophages cultured on type I collagen. These results suggest that the adhesion of macrophages is associated with the tyrosine phosphorylation of a variety of proteins including Lyn and Hck, and that type I collagen serves as a non-adhesive substratum for macrophages, resulting in an altered signal transduction.

Macrophages have a variety of functions in the regulation of cell proliferation, inflammation, and host defense responses against microbial invasion and neoplastic diseases (Adams and Hamilton, 1984). They are distributed in tissues as resident macrophages, such as Kupffer cells in the liver or alveolar macrophages in the lung. On the other hand, circulating monocytes emigrate from the blood stream into sites of inflammation or injury in response to chemotactic stimuli delivered from inflammatory or wounded tissues, to differentiate there into macrophages. In either case, macrophages interact with the extracellular matrix of basement membranes or of intercellular spaces during their differentiation and maturation processes.

The extracellular matrix is composed of a variety of glycoproteins and polysaccharides such as collagen, fibronectin, laminin and proteoglycans. Besides maintaining the tissue architecture, the extracellular matrix plays an important role in regulating functions of various types of cells including immune cells (Juliano and Haskill, 1993). Among fibril-forming collagens (e.g., types I, II, III, V and XI), type I collagen represents the most abundant extracellular matrix in animal tissues, accounting for about one-third of the total protein of the body (Kadler, 1994). Therefore, it is conceivable that immune cells interact with type I collagen when they infiltrate tissue and that their specific immune functions are modulated through interactions with type I collagen. Actually, an integrin-mediated contact of T lymphocytes with type I collagen has profound effects on the aggregation, proliferation, migration, or cytokine secretion of this type of lymphocyte (Sundqvist et al., 1993). A respiratory burst of neutrophils is triggered by their contact with type I collagen through a β2 integrin LFA-1 (Garrotel et al., 1995).

Although the functions of monocytes and macrophages are modulated by fibronectin (Kurowa et al., 1988; Jun et al., 1995), vitronectin (Parker et al., 1988) or laminin (Bohnack et al., 1989), relatively little is known about the effect of type I collagen on the functions of macrophages. In this study, we carried out ultrastructural and biochemical studies on the effect of type I collagen on the adhesion of...
macrophages in vitro.

MATERIALS AND METHODS

Antibodies and reagents

Monoclonal antibodies specific for human β1 integrin (SG/19, IgG1; Seikagaku Corp., Tokyo, Japan), human β2 integrin (IgG1; Upstate Biotechnology Inc., NY, USA), and phosphotyrosine (252 G4, IgG1; Wako, Osaka, Japan) were used. Rabbit antibodies against Lyn, Hck or Fgr were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and horseradish peroxidase-conjugated goat anti-mouse IgG antibody was obtained from Cappel (Durham, NC, USA).

Phorbol 12-myristate 13-acetate (PMA), poly (2-hydroxyethylmethacrylate) (HEMA), phenylmethylsulfonyl fluoride, p-nitrophenyl phosphate, lipopoly saccharide (LPS) (E. Coli 0111: B4), Hoechst 33258 were purchased from Sigma (St. Louis, MO, USA). Na2VO3 and benzamidine hydrochloride hydrate were obtained from Wako, bovine serum albumin (BSA) from Seikagaku Corp., and NaF from Nacalai Tesque (Kyoto, Japan). Pepsin-extracted bovine type I collagen was purchased from Nippi Inc. (Tokyo, Japan). Heat denaturation of type I collagen was carried out by keeping it at 100°C for 3 min. Type III collagen and type V collagen were extracted from bovine placenta with 50 mM acetic acid and 1/100 w/w pepsin in 0.5 M acetic acid, respectively, and purified through differential salting out (Miller and Rhodes, 1982). Fibronectin was purified from bovine serum using a gelatin-Sepharose column (Sakai et al., 1994). Block Ace was purchased from Dainippon Pharm. (Osaka, Japan). A Vectastain mouse ABC kit and DBA substrate kit were obtained from Vector Lab (Burlingame, CA, USA). Protein G-Sepharose and an ECL kit were products of Pharmacia (Uppsala, Sweden) and Amersham (Buckinghamshire, England), respectively.

Adhesion assay

Human monoblastic U937 cells were routinely cultured in RPMI 1640 containing 10% fetal bovine serum (FBS). PMA was dissolved in ethanol at 1 mg/ml and applied to U937 cells at 10 ng/ml to induce their differentiation into macrophages. Twenty-four well tissue culture plates were coated with a 10 μg/ml solution of BSA, type I collagen, heat-denatured type I collagen, type III collagen, type V collagen, or fibronectin at room temperature for 2 h. After washing with PBS, each well was incubated with 1% BSA for 30 min. U937 cells were inoculated to each well at 8×10⁴/ml/well with 10 ng/ml PMA.

On day 1 or 3 of PMA treatment, non-adherent cells were removed by gentle washing and collected by centrifugation. Adherent cells were harvested by treatment with 0.25% trypsin and 0.02% EDTA at 37°C for 10 min. Adherent- and non-adherent cell pellets were suspended separately in 0.5 ml of 0.1% trypsin and 0.02% EDTA and kept at room temperature for 2 h. Each suspension was then mixed with the same volume of 3 mM Hoechst 33258 in PBS and kept at room temperature for 2 h in the dark. Fluorescence was measured with an excitation wavelength at 356 nm and an emission wavelength at 458 nm to determine relative DNA content. Percent of adhesive cells was expressed as \((\{\text{DNA content of adhesive cells}\}/\{\text{DNA content of non-adhesive cells}\}) × 100\).

Electron microscopy

U937 cells were cultured and treated with PMA for 1 or 3 days in the presence of 10% FBS in a plastic culture dish or in dishes coated with protein as described above. After fixation in Karnovsky's fixative at 37°C for 48 h, cells were post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) at room temperature for 1.5 h, dehydrated through an ethanol series, and embedded in Quetol 812 (Nissin EM, Tokyo, Japan). Thin sections were stained with uranyl acetate and lead citrate, and observed under a transmission electron microscope (Hitachi H-700).

Detection of tyrosine phosphorylated proteins

HEMA was dissolved in 90% ethanol at 20 mg/ml and left to dry until coating a dish. Cells (1.5×10⁶) were treated with 10 ng/ml PMA for 6 h in a dish coated with HEMA to prevent cell adhesion to the substratum, washed, and cultured further for 18 h in another HEMA-coated dish without PMA (a total of 24 h). Cells were then transferred to a plastic dish or to dishes coated with HEMA, BSA, type I collagen, heat-denatured type I collagen, or fibronectin and cultured in the absence of FBS for 30 min or 3 h. Both non-adherent cells and adherent cells were lysed with 20 mM Tris (pH 7.4) containing 150 mM NaCl, 1% Triton X-100, 10% glycerol, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na2VO3, 10 mM NaF, 5 mM benzamidine hydrochloride hydrate, and 1 mM p-nitrophenyl phosphate. The lysate was kept on ice for 30 min, and centrifuged at 12,000 rpm for 15 min. The supernatant of the lysate was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (5-20%) under a reducing condition and electrophoretically onto a polyvinylidene difluoride membrane. The membranes were pre-incubated in Block Ace, and probed with an anti-phosphotyrosine mono-
clonal antibody followed by a Vectastain mouse ABC kit. The immunoblots were stained using a DBA substrate kit.

For immunoprecipitation experiments, lysed samples were first agitated with Protein G-Sepharose at 4°C for 2 h. After centrifugation, 1/100 volume of rabbit antibody against Lyn, Hck, or Fgr was added to the supernatant. The immunocomplexes were precipitated by Protein G-Sepharose, and washed thoroughly with the lysate buffer. The immunoprecipitates were boiled in 2x SDS buffer and centrifuged. The supernatant was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10%) under a reducing condition. Separated proteins were then electroblotted onto a polyvinylidene difluoride membrane. After blocking with 1% BSA, the membrane was sequentially treated with anti-phosphotyrosine monoclonal antibody and horseradish peroxidase-conjugated goat IgG anti-mouse IgG, and was visualized using an ECL kit.

Treatment with monoclonal antibody or EDTA
U937 cells were treated with PMA and cultured for 24 h in HEMA-treated dishes as described above. The cells were treated with 10 μg/ml anti-β integrin monoclonal antibody or 5 mM EDTA for the last 30 min of the 24-h culture. Aliquots of the cell suspension were transferred to untreated or type I collagen-coated culture dishes, and the cells were further cultured for 3 h.

Statistical method
Differences in the mean of adherence to the substratum were assessed by Student’s t-test. P<0.05 was considered significant.

RESULTS

Adhesion of macrophages to substratum
The U937 cell is a non-adherent monoblastic cell that differentiates into a mature macrophage after exposure to PMA (GIDLUND et al., 1981; RALPH et al., 1983). Native type I collagen had no significant effect on the growth of U937 cells either in the absence or presence of PMA (data not shown). Upon treatment with PMA, these cells ceased proliferation, adhered firmly, and spread on the plastic substratum (Fig. 1A), BSA (Fig. 1B) or fibronectin (Fig. 1C). In contrast, the adhesion of macrophages to the substratum coated with native type I collagen was markedly reduced on either day 1 or day 3 of PMA treatment (Table 1); the macrophages formed aggregates which were readily removed from the substratum by gentle washings (Fig. 1D). The adhesion of macrophages on the substratum coated with collagens type III or type V was

<p>| Table 1. Inhibition of macrophage adhesion on type I collagen |
|-----------------------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Day</th>
<th>Substratum</th>
<th>Adherence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Plastic</td>
<td>71±2</td>
</tr>
<tr>
<td></td>
<td>Type I collagen</td>
<td>12±1**</td>
</tr>
<tr>
<td>3</td>
<td>Plastic</td>
<td>80±1</td>
</tr>
<tr>
<td></td>
<td>Type I collagen</td>
<td>21±4**</td>
</tr>
</tbody>
</table>

**p<0.001
also weak (Table 2). Adhesion to heat-denatured type I collagen was affected markedly by the presence of FBS; macrophages adhered to some degree to heat-denatured type I collagen in the presence of FBS but not in its absence. This is probably because the serum-derived fibronectin bound to heat-denatured type I collagen and served as an adhesive substratum. Two other types of macrophages—thioglycollate-activated mouse peritoneal exudate macrophages and LPS-treated J774-1 cells which differentiated into macrophages—also adhered weakly to native type I collagen (data not shown).

Table 2. Reduced adhesion of macrophages on fibrillar collagens

<table>
<thead>
<tr>
<th>Substratum</th>
<th>Adherence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastic</td>
<td>74±2 # $ $</td>
</tr>
<tr>
<td>BSA</td>
<td>60±2 * $</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>52±1 ** #</td>
</tr>
<tr>
<td>Type I collagen</td>
<td>26±1 ** # $</td>
</tr>
<tr>
<td>Heat-denatured type I collagen</td>
<td>48±1 ** #</td>
</tr>
<tr>
<td>Type III collagen</td>
<td>19±1 ** # $</td>
</tr>
<tr>
<td>Type V collagen</td>
<td>35±5 ** # $</td>
</tr>
</tbody>
</table>

1) * p<0.05, ** p<0.001; against plastic
2) # p<0.05, ## p<0.001; against BSA
3) $ p<0.05, $ $ p<0.001; against fibronectin

Observation by electron microscopy

Adhesive sites of macrophages cultured in the presence of FBS were observed by transmission electron microscopy. When cells were cultured on the plastic substratum without coating (Fig. 2A) or coated with fibronectin (Fig. 2B), they adhered firmly to the substratum. The lower surface of cells was closely apposed to the substratum at a distance of 14-16 nm. In contrast, when cultured on the substratum coated with native type I collagen, macrophages adhered at their cell processes (Fig. 2C), and the substratum surface showed a mossy plane (about 600 nm thick) of type I collagen microfibrils. Adhesive sites on heat-denatured type I collagen were similar to those on plastic or fibronectin (data not shown).

Detection of tyrosine phosphorylated proteins in macrophages

It has been revealed that the binding of the extracellular matrix to cell surface receptors triggers a signal transduction pathway and induces tyrosine phosphorylation of the receptor or non-receptor proteins, thus inducing or inhibiting the expression of specific genes (Juliano and Haskill, 1993). Therefore, we investigated whether the reduced adhesion of macrophages to native type I collagen is associated with any change in protein tyrosine phosphorylation. In order to avoid the direct effect of PMA on protein tyrosine phosphorylation, U937 cells were first treated with PMA for 6 h in an HEMA-coated dish, washed, then cultured further for 18 h in an HEMA-coated dish in the presence of FBS. They were subsequently plated onto a dish, either uncoated or coated with each extracellular matrix, in the absence of PMA and FBS for 30 min or 3 h. Macrophages did not adhere at all to HEMA (Roskelley et al., 1994) and aggregated. Upon transfer, the adhesion of macrophages under these conditions occurred similarly as shown in Figure 1, though the effect of heat-denatured type I collagen was augmented because of the absence of any serum. As shown in Figure 3A, 35-kDa protein was tyrosine phosphorylated in undifferentiated U937 cells (lane 1). In contrast, many tyrosine phosphorylated proteins were detected in macrophages which adhered onto the plastic surface (lane 2), fibronectin (lane 6) or BSA (lane 7) either at 30 min (Fig. 3A) or 3 h (data not shown) of adhesion. In addition to a major band of 66 kDa, many positive bands were detected around 70 kDa and 60 kDa. In contrast, all macrophages were floating when cultivated on HEMA, and tyrosine phosphorylation was markedly reduced (lane 3). In accordance with the observation that macrophages did not adhere firmly to native or heat-denatured type I collagen, the reduction of tyrosine phosphorylation was also evident on these substrata (lanes 4, 5). These results clearly show that the adhesion of macrophages to the substratum is associated with tyrosine phosphorylation of a variety of proteins, and that type I collagen, either native or heat-denatured, is quite different from fibronectin in both its serving as a non-adhesive substratum for macrophages and reducing protein tyrosine phosphorylation.

Detection of tyrosine phosphorylated Lyn and Hck

We then examined effects of type I collagen on tyrosine phosphorylation of three members of the src gene family, Lyn (53/56 kDa), Hck (56/59 kDa) and Fgr (55 kDa), because reduction of tyrosine phosphorylation was conspicuous for proteins of about 60 kDa, and because the src gene family is involved in the adhesion of cells to the substratum (Erpel and Courtneidge, 1995). As shown in Figure 3B, immunoprecipitation and Western blot analysis clearly showed that both Lyn and Hck were considerably tyrosine phosphorylated in macrophages which ad-
hered to the plastic substratum (lane 2) or the substratum coated with fibronectin (lane 6) or BSA (lane 7). However, their tyrosine phosphorylation was prevented when cultured on HEMA (lane 3), native type I collagen (lane 4) or heat-denatured type I collagen (lane 5). Tyrosine phosphorylation of Fgr was little at best, either in undifferentiated U937 cells (lane 1) or differentiated U937 cells (lanes 2–7). These results suggest that tyrosine phosphorylation of Lyn and Hck is associated with the firm adhesion of macrophages to the substratum, and that cultivation on type I collagen, either native or heat-denatured, results in the marked reduction of the phosphorylation of Lyn and Hck.

**Effect of anti-β integrin monoclonal antibody**

In order to determine whether βα or ββ integrin is involved in the adhesion of macrophages, a blocking monoclonal antibody was added to the culture medium. Although an anti-βα integrin monoclonal antibody inhibited the adhesion of macrophages on fibronectin to some degree and an anti-ββ integrin monoclonal antibody induced the dissociation of cell aggregates, these monoclonal antibodies did not show any effect on the tyrosine phosphorylation of Lyn and Hck (Fig. 3C). These results suggest that neither the epitope of βα integrin nor ββ integrin recognized by these monoclonal antibodies is involved in the adhesion-associated tyrosine phosphorylation of Lyn and Hck in a serum-free medium.

**Effect of EDTA**

The adhesion of macrophages to the plastic substratum seemed to be mediated, at least partly, through the serum- and divalent cation-independent mechanism(s), because macrophages adhered to the plastic substratum in the presence of 5 mM EDTA in a serum-free medium. This adhesion was associated again with the tyrosine phosphorylation of Lyn and Hck (Fig. 3D). Interestingly, this serum- and divalent

**Fig. 2.** Vertical sectional profile of macrophages cultured for 1 day in the presence of 10% FBS and 10 ng/ml PMA on substrata without coating (A), or coated with fibronectin (B) or native type I collagen (C). The arrow indicates the vertical plane of the substratum. Macrophages adhere to type I collagen only at the tips of cell processes (arrowheads). Bar: 1 μm.
cation-independent adhesion mechanism(s) did not work on type I collagen in association with the reduction of the tyrosine phosphorylation of Lyn and Hck.

**DISCUSSION**

We investigated the effect of fibril-forming collagens, particularly type I collagen, on the adhesion of macrophages. In contrast to fibronectin, macrophages adhered to type I collagen weakly at the tip of cell processes, and this was associated with the marked reduction in the tyrosine phosphorylation of a number of proteins, including two members of the src gene family Lyn and Hck.

It is well known that macrophages are highly adhesive to a variety of substrata, including glass and plastic (Adams and Hamilton, 1984). However, Pucillo et al. (1993) reported that U937-derived macrophages adhered weakly on type I collagen. In the present study, we found that U937-derived macrophages did not adhere firmly to the three types of fibril-forming collagen I, III and V. Reduced adhesion on type I collagen is not unique to U937-derived macrophages because a similar effect was also observed with other macrophages such as J774-1 cells and peritoneal exudate macrophages. However, Kaplan and Gaudernack (1982) reported that monocyte-derived human macrophages, when cultured on type I collagen gel, took a bipolar cell shape, aligned parallel to each other, and spread much earlier than on glass. Their observations suggest that monocyte-derived macrophages express some adhesion receptors that facilitate the spreading of macrophages on type I collagen. This apparent discrepancy between monocyte-derived macrophages and the other three types of macrophages used in this study may be explained by different activation states; the former macrophages were differentiated from monocytes without any stimuli, while the other macrophages were stimulated by treatment with PMA or LPS, or

**Fig. 3.** Effects of the substratum on protein tyrosine phosphorylation. A. Tyrosine phosphorylation of total proteins. Lane 1: non-treated U937 cells. Arrowhead indicates a tyrosine phosphorylated 35 kDa band. Lane 2: plastic, lane 3: HEMA, lane 4: native type I collagen, lane 5: heat-denatured type I collagen, lane 6: fibronectin, lane 7: BSA. Molecular mass markers are indicated on the left in kDa. Arrow indicates a 66 kDa band which is tyrosine phosphorylated in adhered macrophages. B. Tyrosine phosphorylation of Lyn, Hck and Fgr. Lanes are described above. C. Effects of an anti-β integrin monoclonal antibody. Macrophages were cultured on plastic (lanes 1, 4, 6), fibronectin (lanes 2, 5, 7) or native type I collagen (lane 3) without a monoclonal antibody (lanes 1–3), with an anti-β integrin monoclonal antibody (lanes 4, 5) or anti-β integrin monoclonal antibody (lanes 6, 7). D. Effects of EDTA. Lane 1: plastic, lane 2: native type I collagen. Arrowheads in B and C indicate heavy chain of IgG.
by i.p. injection of thioglycollate. Actually, monocytes become non-adhesive to type I collagen when they are stimulated with macrophage colony-stimulating factor but adhere to type I collagen when treated with granulocyte/macrophage colony-stimulating factor (De NICHTOLO and BURNS, 1993).

Type I collagen binds to a number of integrin receptors \( \alpha_2 \beta_1, \alpha_2 \beta_3 \) or \( \alpha_2 \beta_1 \). Monocytes bind to type I collagen via \( \alpha_2 \beta_1 \) integrin, suggesting that their adhesion and spreading on type I collagen is mediated, at least partly, by \( \alpha_2 \beta_1 \) integrin (PACIFICI et al., 1992). However, U937-derived macrophages did not express a significant amount of \( \alpha_2 \) integrin subunit (data not shown; ROSKELLEY et al., 1994), though only a small amount of \( \alpha_2 \) was expressed in U937 cells after 4 days of PMA treatment (BAUVOIS et al., 1992). Because U937-derived macrophages adhere weakly to native type I collagen, it seems likely that they did not express collagen receptors which facilitate cell adhesion on type I collagen, though the possibility of the active inhibition of macrophage adhesion by type I collagen can not be completely excluded at present. Taken together, these results suggest that type I collagen is a non-adhesive extracellular matrix for some types of monocytes and macrophages.

Protein tyrosine phosphorylation is a key process in transmembrane signal transduction. Altered protein tyrosine phosphorylation has been observed in macrophages when a ligand binds to Fc\( \gamma \)R (SCHOLL et al., 1992; RANKIN et al., 1993; GREENBERG et al., 1994), an LPS receptor (WEINSTEIN et al., 1991; STEFANOVA et al., 1993) or integrins (STEFANOVA et al., 1993; LIN et al., 1994, 1995). We found that a number of proteins were tyrosine phosphorylated upon adhesion to the substratum and that this tyrosine phosphorylation was markedly reduced when macrophages were cultured on type I collagen. These findings clearly indicate that signal transduction pathways are modulated by type I collagen in association with reduced adhesion to the substratum. It is important to recall that these effects of type I collagen on tyrosine phosphorylation are not unique to U937-derived macrophages, because adhesion-induced tyrosine phosphorylation of a 76 kDa protein in human monocytes is also reduced when they are cultured on type I collagen (LIN et al., 1994).

In the present study, the triple helical structure of native type I collagen seemed to be essential for its serving as a non-adhesive substratum for macrophages, because its activity was reduced to some degree by heat denaturation when tested in the presence of serum. However, in the absence of FBS, macrophages did not adhere to heat-denatured type I collagen, whereas native type I collagen served as non-adhesive substratum regardless of the presence of serum. These observations are explained by the fact that fibronectin, which facilitates the adhesion and spreading of macrophages, binds more firmly to denatured type I collagen than to native type I collagen (ENGVALL and ROUSLAHTI, 1977), resulting in an apparent enhancement of the adhesion to heat-denatured type I collagen in the presence of serum.

The adhesion of macrophages is mediated by a number of cell surface molecules, including \( \beta_2 \) integrin (Mac-1) (ROSEN and GORDON, 1987) or scavenger receptors (FRASER et al., 1993), which do not work without serum and divalent cations. In the present study, however, macrophages adhered well to the plastic substratum without serum and divalent cations. Furthermore, blocking monoclonal antibodies against \( \beta_2 \) integrins did not inhibit their adhesion. These results suggest that these adhesion molecules might work in an unusual manner or that other types of adhesion molecules such as "glass-adherent proteins" (TOMITA and ISHIKAWA, 1992) might be responsible for the adhesion. Although the adhesion of macrophages should be mediated by a number of different mechanisms depending on the state of the macrophages and substratum, the present study suggests that fibrillar collagens—including types I, III, and V—modulate adhesion and signal transduction in macrophages by serving as a non-adhesive extracellular matrix.

The infiltration of immune cells occurs in a time-dependent manner. For example, when the skin is challenged with a hapten, neutrophils appear in the connective tissue of the skin within 12 h, followed by the infiltration of monocyte-derived macrophages (KOYAMA et al., 1996). In chronic contact dermatitis induced by repeated application of a hapten, numerous macrophages appear in the skin in the acute phase of inflammation. However, the number of macrophages decreases and T lymphocytes increase in number in the chronic phase (KUSUBATA et al., 1999). It seems likely that the differential infiltration of the immune cells depends, at least in part, on differential adhesion mechanisms mediated by various components of the extracellular matrix. The present study suggests that type I collagen may play a role in the differential infiltration of neutrophils, macrophages, and lymphocytes in the inflamed tissue because they show different degrees of adhesion to type I collagen.

Type I collagen is known to be enzymatically degraded or denatured at sites of inflammation, and denatured type I collagen binds fibronectin more firmly than the native type I collagen does (ENGVALL and ROUSLAHTI, 1977), thus becoming adhesive to
macrophages. Therefore, it seems likely that macrophages interact with the denatured type I collagen more firmly than with native type I collagen in the inflammatory tissue. It is tempting to speculate that macrophages discriminate and adhere to the denatured type I collagen to degrade and phagocytose it, while they adhere less firmly to undamaged or regenerated native type I collagen. Further studies are required to address this issue.

Acknowledgement. The authors are grateful to Dr. Koko Katagiri for her valuable discussions.

REFERENCES


Dr. Yoh-ichi KOYAMA
Nippi Research Institute of Biomatrix
Nippi Inc.
1-1 Senju-Midoricho, Adachi-ku
Tokyo, 120-8601 Japan
Tel: +81-3-3888-5111
Fax: +81-3-3870-9631
E-mail: tj3y-kym@asihi-net.or.jp

小 山 泰 一
120-8601
東京都足立区千住緑町1-1
（株）ニッピ
バイオマトリックス研究所