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Monocyte, macrophage and foreign body giant cell interactions with molecularly engineered surfaces

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To elucidate the mechanisms involved in monocyte/macrophage adhesion and fusion to form foreign body giant cells on molecularly engineered surfaces, we have utilized our *in vitro* culture system to examine surface chemistry effects, cytoskeletal reorganization and adhesive structure development, and cell receptor-ligand interactions in *in vitro* foreign body giant cell formation. Utilizing silane-modified surfaces, monocyte/macrophage adhesion was essentially unaffected by surface chemistry, however the density of foreign body giant cells (FBGCs) was correlated with surface carbon content. An exception to the surface-independent macrophage adhesion were the alkyl-silane modified surfaces which exhibited reduced adhesion and FBGC formation. Utilizing confocal immunofluorescent techniques, cytoskeletal reorganization and adhesive structure development in *in vitro* FBGC formation was studied. Podosomes were identified as the adhesive structures in macrophages and FBGCs based on the presence of characteristic cytoplasmic proteins and F-actin at the ventral cell surface. Focal adhesion kinase (FAK) and focal adhesions were not identified as the adhesive structures in macrophages and FBGCs. In studying the effect of preadsorbed proteins on FBGC formation, fibronectin or vitronectin do not play major roles in initial monocyte/macrophage adhesion, whereas polystyrene surfaces modified with RGD exhibited significant FBGC formation. These studies identify the potential importance of surface chemistry-dependent conformational alterations which may occur in proteins adsorbed to surfaces and their potential involvement in receptor-ligand interactions. Significantly, preadsorption of α_2-macroglobulin facilitated macrophage fusion and FBGC formation readily on the RGD surface in the absence of any additional serum proteins. As α_2-macroglobulin receptors are not found on blood monocytes but are expressed only with macrophage development, these results point to a potential interaction between adsorbed α_2-macroglobulin and its receptors on macrophages during macrophage development and fusion.

These studies identify important surface independent and dependent effects in foreign body reaction development that may be important in the identification of biological design criteria for molecularly engineered surfaces and tissue engineered devices.

1. Introduction

Tissue-engineered devices may require a biodegradable or non-biodegradable synthetic substrate which provides a construct or framework for the proposed cellular components. The biocompatibility of these substrates, with or without cells, is an important consideration in the design of tissue-engineered devices. It can be anticipated that when these constructs are implanted into vascularized connective tissue, which is present in virtually all organs and tissues, an inflammatory response followed by a wound healing response with development of the foreign body reaction, i.e. macrophages and foreign body giant cells (FBGC), at the tissue/substrate interface will occur. To better understand the mechanism of formation of the foreign body reaction and its potential role in the success or failure of tissue-engineered devices, we have carried out studies on lymphokine-induced foreign body giant cell formation on molecularly engineered substrates. These studies address the hypothesis that the monocyte/macrophage (MC/MO) is the major cellular component controlling the tissue/material inflammatory and healing responses and that molecularly engineered substrates can selectively affect the activation, function and cytokine production of tissue macrophages.

Fig. 1 illustrates the sequence of events and the inflammatory response to implants and significant variables which may modulate protein adsorption, monocyte adhesion, macrophage development and

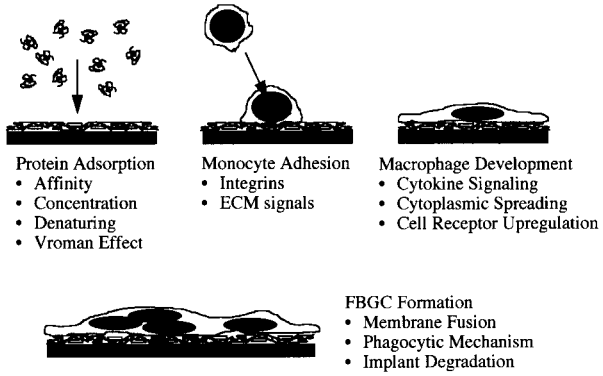


Figure 1 The inflammatory response at tissue/material interfaces.

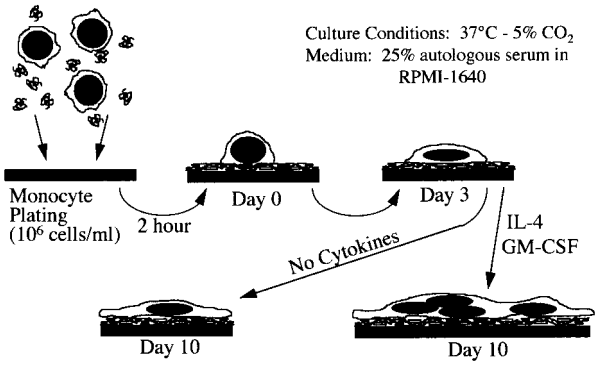


Figure 2 The *in vitro* protocol for foreign body giant cell formation on molecular engineered surfaces.

foreign body giant cell formation. It must be remembered that this sequence of events is a continuum with one event overlapping the next event in the progression of the inflammatory and wound healing responses.

We have recently demonstrated that differential lymphokine regulation of macrophage fusion leads to morphological variants of multinucleated giant cells (FBGC) [1, 2]. Fig. 2 and Table I illustrate and provide detailed information on the *in vitro* conditions utilized to generate foreign body giant cells on molecularly-engineered surfaces. Interleukin-4 (IL-4) and interleukin-13 (IL-13) have been shown to independently promote monocyte/macrophage fusion to form FBGC, whereas interferon-γ has been demonstrated to form Langhans-type giant cells. IL-4 and IL-13 are secreted predominantly by T helper 2 (Th2) lymphocytes and suggest a role for lymphocytes, present during the transient chronic inflammatory response, in the development of foreign body giant cells. Fig. 3 shows typical *in vitro* results for foreign body giant cell formation on molecularly engineered surfaces. The role for IL-4 in FBGC formation was confirmed with *in vivo* studies using the rat cage implant system and a polyurethane together with IL-4 neutralizing antibodies and recombinant murine IL-4 [3]. Participation of the macrophage mannose receptor (MMR), upregulated by IL-4 or IL-13, was demonstrated with confocal immunofluorescent studies [4]. IL-4-induced macrophage fusion and giant cell formation was prevented by competitive inhibitors of MMR activity, i.e. α-mannan, or inhibitors of glycoprotein processing that restrict MMR surface expression [4].

This paper presents our more recent efforts to develop an in-depth understanding and an elucidation of the mechanisms which are involved in macrophage fusion and foreign body giant cell formation on surfaces. We have examined surface chemistry effects, cytoskeletal reorganization and adhesive structure development, and cell receptor–ligand interactions in *in vitro* foreign body giant cell formation.

2. Surface chemistry effects on *in vitro* FBGC formation

Previous studies in our laboratory have suggested that the formation of FBGCs may be dependent upon substrate chemistry [1]. To confirm this phenomenon, we utilized silane surface modification of glass coverslips to produce a wide variety of chemistries for use as culture substrates in our FBGC formation culture protocol. Results from these studies demonstrated that surface chemistry can greatly affect both macrophage adhesion and FBGC formation.

The surfaces under investigation were produced by our laboratory using the well documented silane modification of glass [5]. Briefly, glass coverslips are cleaned in subsequent NaOH and HNO_3 solutions. The clean and dry coverslips are immersed for 5 min in a 5% silane/chlorobenzene solution or 1 min in a 1% silane/ethanol solution and dried. Fig. 4 shows the chemical reactions involved in the binding of a generic R-group trifunctional silane to a glass surface and is representative for all silanes [6]. Table II displays the silane functional group

TABLE I Culture protocol for IL-4-induced FBGC formation

DAY 0	Isolate human blood monocytes by a non-adherent, density centrifugation method and suspend at 1×10^6 cells/ml in RPMI-1640 containing 25% autologous serum. Add 5×10^5 monocytes to each well (24-well plate) containing a 13 mm diameter silane modified glass coverslip, held down by a silicon rubber ring (9 mm internal diameter). Allow monocytes to adhere for 2 h at 37 °C in humidified incubator (5% CO_2). Aspirate medium and wash wells with PBS (with Ca^{2+} and Mg^{2+}, 37 °C). Replace with 1 ml/well RPMI containing 25% autologous serum, and return to incubator.
DAY 3	Aspirate old medium and replace with RPMI containing 25% heat-treated (56 °C for 1 h) autologous serum, and add either: (1) no cytokines (2) IL-4 (10 ng ml^{-1}) and GM-CSF (10 ng ml^{-1})
DAY 7	Repeat as on day 3
DAY 10	Discard medium, wash wells 2 × with warmed PBS (with Ca^{2+} and Mg^{2+}, 37 °C), and fix wet cells with methanol for 5 min. Air-dry and stain with May–Grünwald/Giemsa.

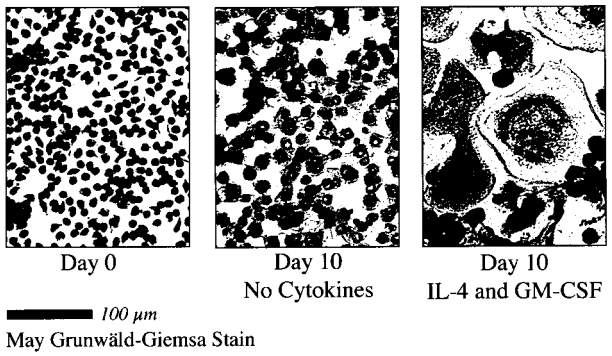


Figure 3 Typical *in vitro* results from monocyte/macrophage culture on molecular engineered surfaces which facilitate FBGC formation.

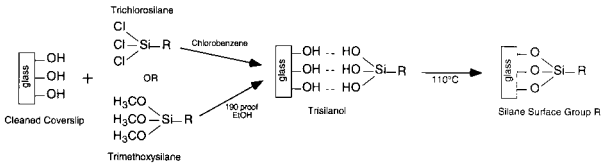


Figure 4 Binding of trifunctional silane to glass surface.

(R) and abbreviation for each surface prepared during this investigation. While the majority of surfaces were produced by selecting specific silane reagents which contained the desired functional group, the COOH, OH and Br surfaces were prepared by modifying the vinyl surface double bond as described by Wasserman *et al.* [7]. Analysis of all surfaces included advancing water contact angle and elemental composition by X-ray photoelectron spectroscopy (XPS).

Utilizing our protocol for IL-4-induced FBGC formation (Fig. 2 and Table I), we evaluated the ability of each of the 14 surfaces to inhibit or enhance monocyte/macrophage adhesion and macrophage fusion. Monocyte/macrophage adhesion was quantified by optical microscopy at 2-h and 10-day time points and expressed as the fraction of originally plated cells which remained adherent. FBGC density (cells/mm^2) and FBGC size (mm^2) were measured from day 10 cells that had been exposed to a combination of IL-4 and granulocyte–macrophage colony-stimulating factor (GM-CSF).

This experiment revealed that monocyte/macrophage adhesion over a 10-day culture was essentially unaffected by surface chemistry, however the density of IL-4-induced FBGCs could be correlated to surface carbon content (Fig. 5). The only exception to the surface-independent macrophage adhesion were the alkyl-silane modified surfaces. DM and TD, which exhibited reduced adhesion and FBGC formation, prompted further investigation into this class of surfaces. We hypothesize that the specific silane functional group is not crucial to the modulation of fusion. Rather, we believe that the amount of carbon present in the surface layer is an indicator of macrophage fusion potential. The mechanisms of this phenomenon are not clear, but are likely mediated by adsorbed protein-adhesion receptor interactions which are currently under investigation.

Literature reports of cell adhesion on alkyl-silane modified surfaces have demonstrated that these surfaces generally inhibit cell adhesion, although no reports utilizing human monocytes or macrophages are available [8–11]. The low monocyte/macrophage adhesion levels we observed on DM and TD surfaces are consistent with these reports, however the FBGC formation data on these two surfaces were inconclusive. To further explore the cellular response to alkyl-silane modified glass, we initiated an in-depth study examining macrophage adhesion and fusion on DM, TD, as well as methyltrichlorosilane-modified glass (C1) and octadecyltrichlorosilane-modified glass (C18).

Synthesis and characterization of these surfaces (Table II) were carried out as described above, except that the methyltrichlorosilane was handled under nitrogen to eliminate aggregation in solution. Additionally, all surfaces were sonicated in ethanol immediately after silane modification to remove any aggregate or multilayer formation.

When compared to the hydrophilic clean glass, the alkyl-silane surface modifications resulted in hydrophobic surfaces (Fig. 6) with contact angles of $\sim 70^\circ$ for methyl surfaces (C1 and DM) and $\sim 100^\circ$ for the longest alkyl-chains (TD and OTS). As expected, increased

TABLE II Silane modified surfaces

Surface abbreviation	Surface functional group (R)	Contact angle (H_2O)	%Si	%O	%C	Other
AP	$-(CH_2)_3NH_3^+$	47.2±6.0	19.0	44.6	29.2	7.2 N
Br	$-CHBrCH_2Br$	95.6±6.5	31.9	55.2	9.7	3.3 Br
COOH	$-COO^-$	13.6±3.1	24.5	62.0	12.6	
Clean	$[-OH]^a$	11.0±2.2	23.4	59.9	13.6	
DA	$-(CH_2)_3NH_2^+CH_2CH_2NH_3^+$	13.7±3.0	21.0	48.7	24.4	5.9 N
DM	$-CH_3$	66.9±3.2	26.9	57.5	14.4	
OH	$-CH_2CH_2OH$	3.2±0.9	25.9	62.1	12.0	
Phenyl	$-C_6H_5$	73.3±2.6	23.4	47.9	28.7	
TD	$-(CH_2)_{13}CH_3$	100.9±2.1	16.7	32.5	50.8	
TA	$-(CH_2)_3NH_2^+CH_2CH_2NH_2^+CH_2CH_2NH_3^+$	12.5±4.6	18.4	45.9	27.0	8.8 N
SiH	$-H$	108.0±4.6	34.4	61.5	4.0	
13F	$-CH_2CH_2(CF_2)_5CF_3$	102.9±2.9	6.6	10.9	29.7	52.8 F
3F	$-CH_2CH_2CF_3$	75.9±3.8	21.9	42.4	21.9	13.8 F
Vinyl	$-CH=CH_2$	118.3±2.7	36.0	58.9	5.2	

[a]No silane was grafted to the clean glass surface, however high densities of silanol hydroxyl groups are normally present. The culture environment (pH 7.3) is believed to ionize both the amine and carboxyl groups.

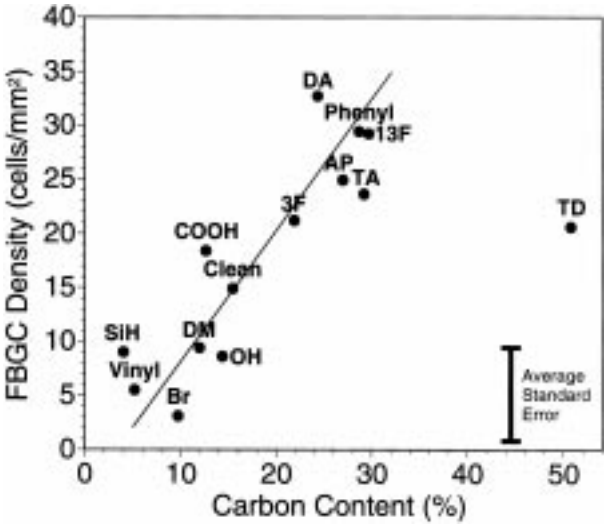


Figure 5 The density of day 10 FBGCs induced by IL-4 and GM-CSF has a positive relationship with surface carbon content as measured by XPS. Linear regression (solid line) resulted in r^2 values of 0.82 for IL-4 and 0.79 for IL-4/GM-CSF data. The TD surface was not included in the regression analysis due to an atypical carbon content. Each point on the plot represents data for a single surface which is identified by the adjacent label. Error bars for each data point are omitted to reduce clutter, however the average standard error of FBGC density is included ($n = 3$).

carbon levels and decreased oxygen and silicon levels (Fig. 7) were observed for the longest alkyl chain surfaces (TD and OTS). Interestingly, the clean glass, DM, and C1 surfaces all displayed similar carbon levels, possibly as a result of contamination by atmospheric hydrocarbons.

After characterization we began a series of four identical monocyte cultures to analyze monocyte/macrophage adhesion and FBGC formation on alkyl-silane modified surfaces. An additional adhesion time point at day 3 was included. The adhesion results (Fig. 8) showed that initial adhesion (2 h) was similar for all surfaces regardless of chemistry. However, by day 3 and day 10, the long-chain alkyl-silane surfaces (TD and OTS) nearly eliminated macrophage adhesion. TD and OTS also eliminated the presence of IL-4-induced

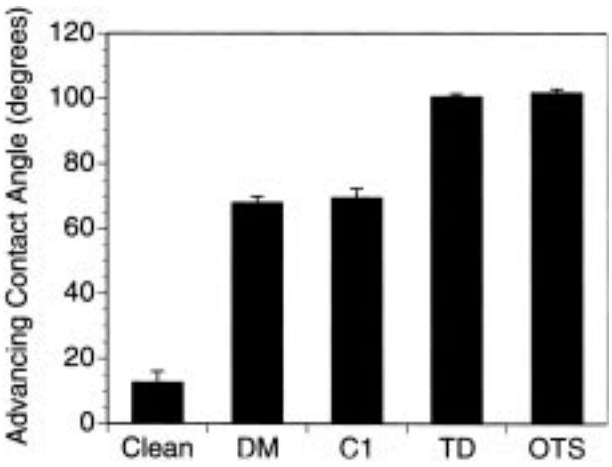


Figure 6 Water contact angle for alkyl-silane modified glass surfaces: clean glass, DM, C1, TD and OTS. Data points are expressed as mean values with error bars representing standard deviation.

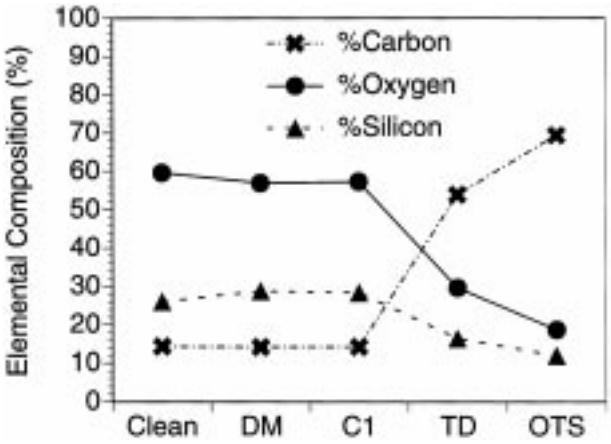


Figure 7 XPS elemental composition for alkyl-silane modified glass surfaces: clean glass, DM, C1, TD and OTS. Calculated from high resolution Si2p, O1s, N1s, C1s scans.

FBGCs by day 10 (Fig. 9). Those surfaces with shorter alkyl chains (DM and C1) appeared to have indistinguishable adhesion throughout the 10-day culture, in addition to similarly high levels of IL-4-induced FBGC formation. We reasoned that the low adhesion levels could account for the low FBGC levels (i.e. TD and OTS) because FBGC formation requires close contact of macrophages. In the case of the clean glass, the macrophage density was high enough to allow maximal levels of FBGC formation, however negligible FBGC formation was observed. Most likely the composition and conformation of the proteins adsorbed on the clean glass surface signalled the adherent macrophages to down-regulate the processes of FBGC formation. This study of alkyl-silane modified glass has demonstrated that both long-term macrophage adhesion and IL-4-induced FBGC formation are surface dependent phenomena.

Using our human monocyte culture protocol which has proven to reliably induce macrophage fusion, we have tested a variety of silane-based surface chemistries to confirm the surface dependence of both monocyte/

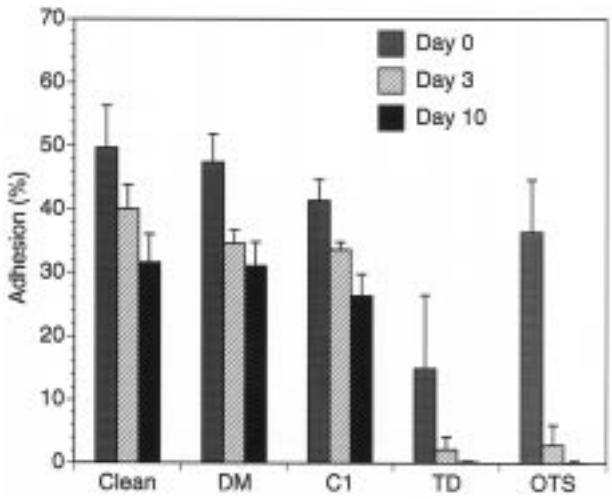


Figure 8 Day 0, 3 and 10 monocyte/macrophage adhesion on alkyl-chain silane modified glass surfaces. Adhesion expressed as the percentage of originally plated monocytes still adherent. All values expressed are averages with error bars representing standard error values.

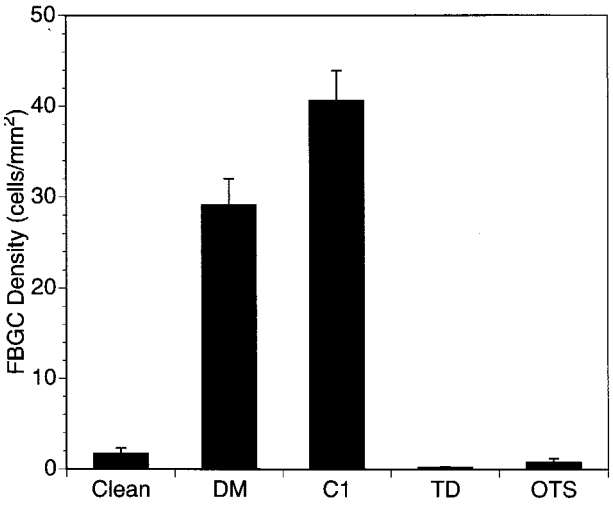


Figure 9 Day 10 FBGC density on alkyl-chain silane modified glass surfaces. All values expressed are averages with error bars representing standard error values.

macrophage adhesion and FBGC formation. The initial panel of 14 silane-based surfaces identified surface carbon content as an important indicator of potential for macrophage fusion. A group of alkyl-silane surfaces revealed that methyl surfaces allowed abundant adhesion and high levels of FBGC formation, while the longer alkyl chains inhibited both adhesion and FBGC formation. Interestingly, clean glass allowed high levels of adhesion, but inhibited FBGC formation. These experiments have identified three types of surfaces: DM and C1 which allow high levels of adhesion and FBGC formation, TD and OTS which allow neither adhesion nor FBGC formation, and clean glass which allows adhesion but no macrophage fusion. This type of surface dependence is likely mediated by ligands in the adsorbed protein layer which can bind the adhesion receptors of adherent cells thereby modulating cellular behavior.

3. Cytoskeletal reorganization and adhesive structure development in *in vitro* FBGC formation

During the foreign body reaction, monocytes undergo a striking phenotypic progression of differentiation into macrophages which may subsequently fuse to form FBGC. The dramatic morphological alterations that occur during monocyte maturation and macrophage fusion must be supported by cytoskeletal reorganization and may indicate the acquisition of macrophage functional capabilities. Cytoskeletal participation has been demonstrated for many monocyte/macrophage functions that are critical during the inflammatory response, including migration [12], attachment and adhesion [12–14], spreading [13, 15], phagocytosis [15, 16] and secretion [17]. Using our *in vitro* system of cytokine-induced FBGC formation on silane-modified coverslips in conjunction with confocal fluorescence microscopy, the cytoskeletal reorganization and adhesive structure development in monocytes, macrophages and FBGC were investigated (Fig. 10).

Human monocytes were cultured for 10 days on either dimethylsilane-treated or untreated coverslips. On days 3 and 7, IL-13 was added to some culture wells. In Fig. 10, the left column (a, c, e) shows F-actin staining with rhodamine phalloidin. The right column (b, d, f) shows tubulin staining by indirect immunofluorescence. Optical slices were collected with a confocal microscope and were projected to create the final pictures. Fig. 10a and b show macrophages cultured on dimethylsilane without the addition of exogenous cytokines. Cells have punctate F-actin across the ventral cell surface and highly branched microtubule structures. Fig. 10c and d show FBGC that has formed in response to IL-13 on a dimethylsilane coverslip. The punctate F-actin is restricted to the extreme periphery of the cells and the microtubules can be seen surrounding circular structures (unstained nuclei). Fig. 10e and f show macrophages treated with IL-13 and cultured on untreated coverslips. Macrophage fusion does not occur, and many cells take on a very elongated morphology. Punctate F-actin is visible at each end of the elongated cells, and microfilaments and microtubules span the long axis of the cells.

Monocytes contained linear filamentous F-actin, mostly concentrated in membrane ruffles, that redistributed into intensely staining punctate foci on the substrate-attached side of adherent cells during macrophage development (Fig. 10a). Linear F-actin was visible along the plasma membrane throughout the volume of the cell. Spread cells contained a centrally located concentration of tubulin from which highly branched and interconnected microtubules radiated towards and parallel to the cell periphery (Fig. 10b).

Inasmuch as IL-13 has been demonstrated to induce macrophage fusion and FBGC formation [2], cultures were treated with IL-13 in order to examine the cytoskeleton and adhesive structures of these multinucleated macrophages. In contrast to macrophages, punctate F-actin at the ventral cell surface was restricted to the extreme periphery of FBGC and was very dense (Fig. 10c). FBGC contained dense meshworks of microtubules that generally formed circles concentric to the plasma membrane (Fig. 10d). Microtubules were present in the FBGC periphery but did not terminate in the podosomes where punctate F-actin structures were concentrated.

Interestingly, we found that IL-13 induced a dramatic change in macrophage morphology on culture surfaces that did not support macrophage fusion and FBGC formation. On coverslips not treated with any silane, IL-13 did not induce FBGC formation although macrophage adhesion was comparable to that on silane-treated coverslips. Instead, macrophages assumed an elongated, spindled morphology. Where spindles terminated on the coverslip surface, punctate F-actin structures formed (Fig. 10e). Microfilaments and microtubules extended along the long axis of these macrophages (Fig. 10e and f).

Because the monocytes were subjected to otherwise identical culture conditions, these surface-dependent morphological responses may be explained by quantitative differences in the adsorption of serum proteins and/or by adsorption-induced conformational alterations of potentially important adhesion proteins, such as fibronectin and vitronectin. This is supported by observations

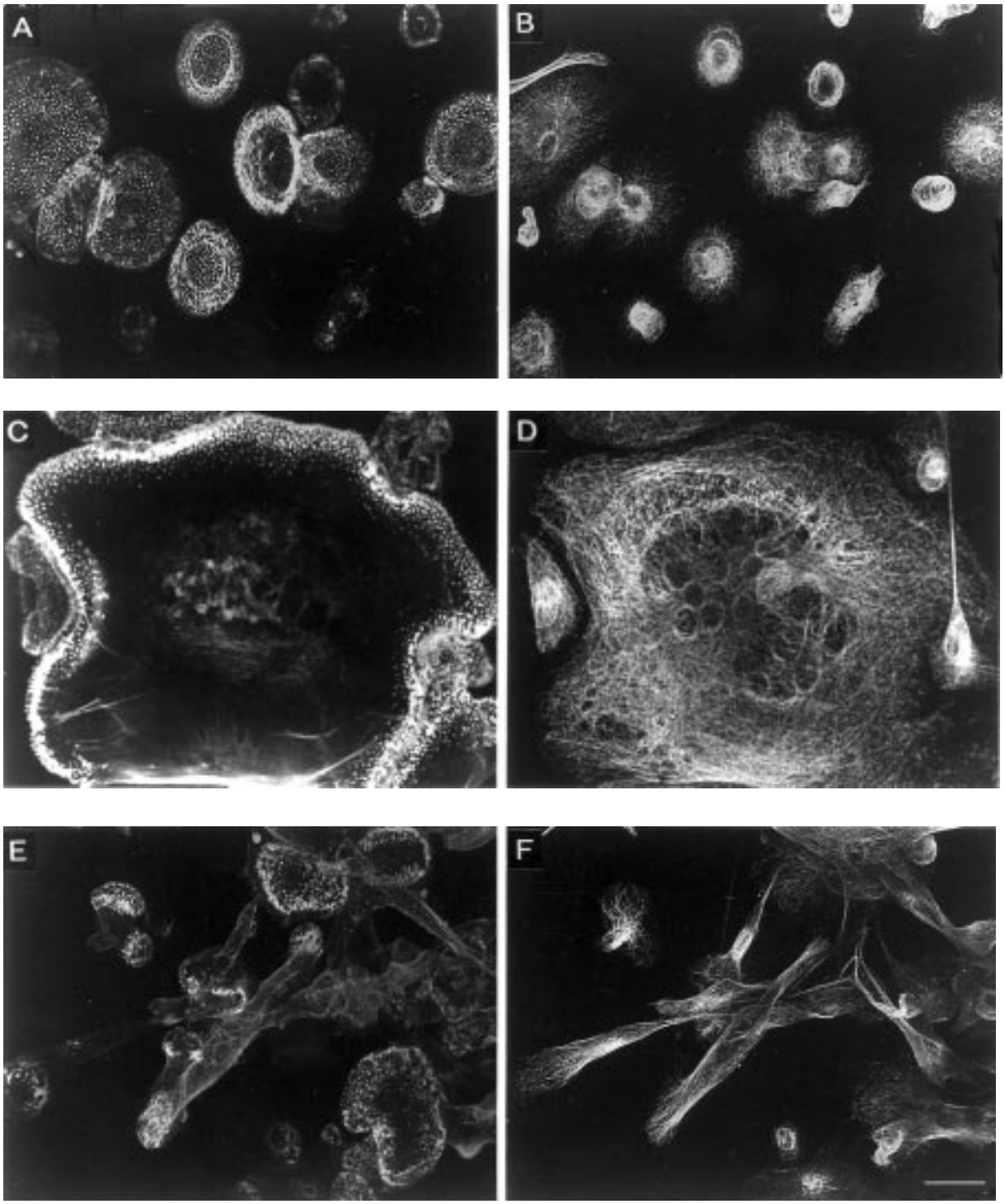


Figure 10. Effect of surface chemistry and cytokine treatment on macrophage microfilament and microtube orginisation. Human monocytes were cultivated for 10 days on dimethylsilane-treated (a–d) or untreated (e–f) coverslips and were stained for F-actin (a, c, e) and tublin (b, d, f). Progression to a macrophage phenotype occurred (a, b) and punctuate F-actin was visible (a, c, e). Interleukin-13 was added on days 3 and 7 of culture, which induced fusion to form FBGC (c, d) or a morphological change to elongated macrophages without fusion (e, f). Scale bar = 25 μm.

that fibroblasts and neural cells rely on different binding activities of fibronectin adsorbed to chemically distinct surfaces to achieve their cell-type- and surface-specific phenotypes [18]. Cell spreading and differentiation were significantly different on chemically distinct surfaces as were the development of stress fibers and focal contacts, which are indicators of subsequent fibroblast activities such as movement, mitosis and gene expression [19]. Therefore, these results suggest that the underlying substrate is critical to the acquisition of cell shape, responsiveness to stimuli and subsequent functional specializations.

The punctate F-actin fluorescence along the ventral cell membrane is indicative of adhesive structure formation at these sites (Fig. 10a, c, e) [20, 21]; however, the type of adhesive contact cannot be identified by F-actin staining alone. Table III summarizes our efforts to characterize these areas and to more thoroughly describe the composition of adhesive structures that are acquired during macrophage development. Macrophages and IL-

TABLE III Macrophage and FBGC podosome protein organization

Protein	Ring-like structure around actin core	Co-localization with actin core
Vinculin	+	−
Talin	+	−
Paxillin	+	−
Gelsolin	−	+
L-plastin	−	+
FAK	−	−

13-induced FBGC were fixed with formaldehyde on day 10 of culture. Cells were stained for F-actin with rhodamine-phalloidin and for one of the listed cytoplasmic proteins by indirect immunofluorescence. Cells were viewed with a confocal microscope and double label images were collected from the ventral cell surface. Vinculin and talin are well-described adhesive structural proteins. Talin is thought to link integrins to vinculin which binds α-actinin, an actin binding protein. Paxillin is a more recently described protein that is thought to be brought to focal contacts by focal adhesion kinase (FAK). In macrophage podosomes, paxillin is present without FAK. Gelsolin is an actin capping protein that may explain why stress fibers do not form from podosomes but do from focal contacts (which do not contain gelsolin). L-plastin (fimbrin) is an actin-bundling protein which localizes to the phagocytic cup in phagocytosing macrophages. The organization of the cytoplasmic proteins shown in the table supports identification of macrophage and FBGC adhesive structures as podosomes.

Upon development of punctate F-actin structures, vinculin, talin and paxillin formed ring-like structures around the F-actin but did not co-localize with it. The lack of co-localization between F-actin and these structural proteins excluded their identification as focal contacts or close contacts. However, the arrangement of vinculin, talin and paxillin in ring structures surrounding F-actin cores is consistent with podosome adhesive structures [13, 20]. For further confirmation, cultures were stained for gelsolin, which also has been described to be a component of podosomes [20] but not focal or close contacts. Gelsolin organized into punctate structures that co-localized with F-actin along the ventral cell surface of macrophages. L-plastin, or fimbrin, which has been described in mouse macrophages [22], also co-localized with punctate F-actin.

The presence of paxillin prompted investigation for the presence of FAK, the activation of which has been described as an important step in the recruitment of paxillin to developing adhesive structures in rat embryo fibroblasts and mouse 3T3 cells [23]. However, in agreement with published reports, FAK was not detected in monocytes or macrophages at any time during the 10-day culture period [24].

The presence of a particular type of adhesive structure may reveal specific macrophage functional activity at cell/material interfaces. Podosomes may enable a macrophage to mobilize membrane-associated proteases [25] and/or other components to the ventral cell surface. Further, podosome spatial alterations during FBGC formation may imply additional functional polarizations. This may reflect frustrated phagocytosis [26] via the formation of a closed compartment [27, 28] between FBGC and the culture material surface into which degradative enzymes and/or other products are secreted. This possibility is consistent with the enrichment of a lysosomal antigen at the ventral cell surface of FBGC as compared to mononuclear macrophages [29] and with the occurrence of material surface cracking, an indicator of degradation, directly beneath adherent FBGC but not beneath mononuclear macrophages [30].

Extensive cytoskeletal and adhesive structural polarizations occur in monocytes during their phenotypic progression to macrophages and following subsequent cytokine-induced macrophage fusion to form FBGC. Further, the adhesive structures of macrophages and FBGC were identified as podosomes based on the presence of characteristic cytoplasmic proteins and F-actin at the ventral cell surface. Importantly, these studies provide the first description of the cytoskeletal and adhesive structure organization in human cytokine-induced FBGC and enhance our understanding of macrophage and FBGC cytoskeletal and adhesive structural support, which is likely critical for the acquisition of functional specializations by these cells in the foreign body reaction.

4. Macrophage receptor–ligand interactions in *in vitro* FBGC formation

Monocytes/macrophages express a variety of receptors on their cell membranes which have the potential to mediate adhesion to adsorbed blood proteins. Among these are receptors for the adhesion proteins fibronectin and vitronectin, which contain the arginine–glycine–aspartate (RGD) cell recognition sequence, as well as for complement components and immunoglobulins. We previously observed that certain receptors for complement component C3 are important for initial monocyte adhesion to differently modified polystyrenes [31], but that these interactions diminish and are apparently replaced by others during monocyte-to-macrophage development and FBGC formation *in vitro*. Using a variety of functionally inhibitory antibodies to several different receptors for fibronectin, we also find that fibronectin (Table IV) or vitronectin (data not shown) do not play major roles in initial monocyte adhesion.

Using our *in vitro* system of lymphokine-induced FBGC formation, however, we have recently discovered that FBGC formation proceeds much more readily on polystyrene surfaces to which the RGD tripeptide has been covalently attached than on polystyrene culture

TABLE IV Effects of antibodies[a] to fibronectin receptors on monocyte adhesion[b] to various modified polystyrene surfaces[c]

Experiment	Anti-	PA	PB	PC	PM
A	α3	84±1	97±4	98±2	105±10
	gpIIIa	78±8	91±4	93±6	97±4
	α5β1	67±11	76±6	98±6	82±5
B	β1	106±3	117±9	122±5	112±14
	α4	114±13	106±3	104±3	97±6
	gpIIIa/IIB	110±3	108±4	116±3	113±1
	IgG control	103±6	111±1	111±3	112±8

[a]Anti-α3, nude mouse ascites; anti-gpIIIA, purified mouse immunoglobulin G (IgG) (AMAC); anti-α51, rabbit serum; anti-1, nude mouse ascites; anti-α4, nude mouse ascites; anti-gIIIa/IIb affinity-purified IgG; IgG control, affinity purified IgG.
[b]Expressed as the per cent of values obtained without antibodies as previously described [32].
[c]From Mat Tek Corp. and modified as follows: Plastek A (fluorinated), Plastek B (siliconized), Plastek C (nitrogenated), Plastek M (oxygenated).

TABLE V Comparison of macrophage fusion[a] on polystyrene versus RGD-modified polystyrene. Effect of the addition of serum

Surface	% Autologous serum added[b]			
	0	1	5	10
PS[c]	3±3	75±5	78±9	73±3
RGD[d]	99±1	63±5	83±4	88±2

[a]Macrophage fusion values represent the percentage of nuclei in multinucleated foreign body giant cells compared to the total numbers of nuclei counted in three 10× objective fields.
[b]Monocytes were plated in 20% autologous serum [1].
[c]Polystyrene Plastek M (Mat Tek Corp.).
[d]RGD-modified polystyrene (Chemicon).

material (Table V). In this case, the surfaces were exposed to serum during cell plating, after which soluble serum components were removed by washing. On polystyrene, there is no requirement for RGD if serum is also present in the culture medium during the induction of fusion (Table V). These data suggest that, in addition to the RGD sequence, there is a component(s) in serum which is necessary for fusion to proceed and which can apparently be presented to the monocyte/macrophage in the solid phase together with immobilized RGD.

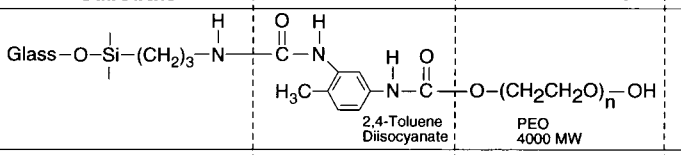
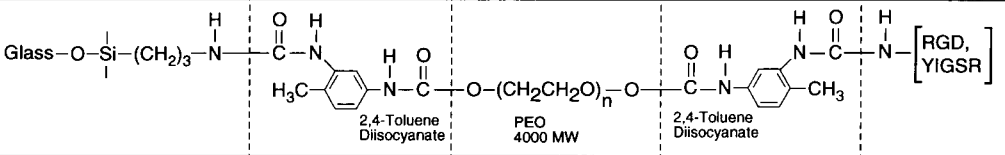
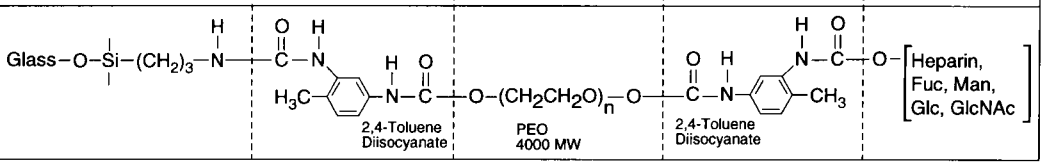
In order to pursue the identity of this protein, we pre-adsorbed RGD surfaces with either albumin, fibronectin, vitronectin or α_2-macroglobulin, which is a prominent serum protein for which macrophages also express receptors and which has been implicated in oxidative stress cracking on polymer surfaces [32]. Monocytes were then allowed to attach in the absence of any added serum, and fusion was induced in the presence or absence of 20% serum. As is shown in Table VI, the required serum component is unlikely to be fibronectin or vitronectin as fusion cannot proceed on fibronectin- or vitronectin-adsorbed RGD-modified polystyrene without the addition of serum. FBGC formation also does not occur on fibronectin- or vitronectin-adsorbed unmodified polystyrene (data not shown). Very limited fusion does occur if albumin is adsorbed, but this may reflect the purity of commercially available albumin, which is a well known carrier protein. However, if α_2-macroglobulin is used for pre-adsorption, macrophage fusion occurs readily on the RGD surface in the absence of any additional serum proteins. As α_2-macroglobulin receptors are not found on blood monocytes but are expressed only with macrophage development [33], these data point to a potential interaction between

TABLE VI The effect of pre-adsorption of RGD-modified polystyrene with various serum proteins on macrophage fusion[a] in the presence or absence of 20% serum

Pre-adsorbed protein[b]	Autologous serum added during fusion[c]	
	No	Yes
0	0	83±3
Albumin	21±9	82±1
Fibronectin	0	83±2
Vitronectin	0	78±7
α_2-Macroglobulin	92±1	79±2

[a]Macrophage fusion values represent the percentage of nuclei in multinucleated foreign body giant cells compared to the total numbers of nuclei counted in three 10× objective fields.
[b]RGD-modified culture wells were pre-adsorbed with each protein at 20% of its normal serum concentration in phosphate-buffered saline, pH 7.4, for 30 min at 37 °C, washed three times to remove unadsorbed protein, and immediately seeded with freshly isolated monocytes. Fibronectin, α_2-macroglobulin, vitronectin and human serum albumin.
[c]Monocytes were plated and allowed to attach in serum-free medium [1].

TABLE VII Molecular engineered surfaces.

Surface Identification	Aminopropylsilane Substrate	Link	Spacer or Pendant Group	Link	Pendant Group
PEO Glass					
Peptide Glass					
Heparin and Carbohydrate Glass					

adsorbed α_2-macroglobulin and its receptors on macrophages during macrophage development and fusion.

Therefore, there are apparently a minimum of two types of adhesive interactions which are required for macrophage fusion leading to FBGC formation *in vitro*. The first probably involves an RGD-containing protein, the identity of which is as yet unclear. The participation of fibronectin or vitronectin cannot be ruled out because it is possible that material surface-chemistry-dependent conformational alterations occur in these native proteins during adsorption that may render the RGD and/or other relevant cell attachment sites unavailable to interact with cell receptors.

The second adhesive interaction may involve α_2-macroglobulin, which is an abundant plasma protein that can form complexes with a variety of proteinases. Thus, it is believed to function in plasma clearance of these deleterious substances [34]. In this regard, it is of interest that the binding of α_2-macroglobulin to its cell receptor does not occur unless it is complexed with a proteinase, thus inducing a conformational change in the molecule and exposing receptor-binding domains. Inasmuch as macrophage fusion occurred in our culture system on α_2-macroglobulin-adsorbed RGD polystyrene without the addition of any other exogenous proteins, the possibility is raised that proteinases secreted by the macrophages themselves may complex with α_2-macroglobulin and enable an interaction with the α_2-macroglobulin receptor. If this is the case, the formation of FBGC on implanted biomaterials may represent an ongoing down-modulation of the inflammatory response by macrophage sequestration of deleterious proteinases from surrounding host tissues.

5. Future perspectives

The overall goal of our research is to develop an understanding of the mechanisms of monocyte/macrophage adhesion, macrophage activation, macrophage fusion to form foreign body giant cells, and foreign body giant cell function on implanted biomaterial surfaces. Information and perspectives gained from these studies will provide biologically derived design criteria for surface modification of substrates which will modulate these cell/surface interactions in a fashion appropriate to the desired tissue engineered device or molecularly engineered surface. Studies presented in this work illustrate the surface-dependent and -independent interactions which may occur in the development of foreign body giant cells on surfaces. Long-chain alkyl modification of surfaces may prove to be a desired modification of devices when inhibition of the foreign body reaction is desired. Our cytoskeletal reorganization and adhesive structure development studies demonstrate that macrophages and foreign body giant cells, unlike fibroblasts, do not form focal adhesions and stress fibers. We believe these results are related to the innate mobility of the cell. Moreover, they provide information important to future studies on signal transduction mechanisms involving integrins and other receptors on macrophages and foreign body giant cells which may interact with protein adsorbed to the molecularly engineered surfaces. Pre-adsorbed proteins on surfaces may alter the conformation of the protein and modulate the interaction of cell receptors with pre-adsorbed protein ligands. While monocytes do not utilize RGD receptors in early adhesion events, phenotypic progression of monocytes to macrophages result in an apparent upregulation of RGD receptors on macrophages. The phenotypic modulation of receptor expression from monocytes to macrophages to foreign body giant cells is currently under investigation. Table VII illustrates the various types of surface chemistries which currently are being investigated in these studies. The utilization of polyethylene oxide (PEO) spacer molecules together with the diisocyanate coupling technique has permitted a wide variety of pendant groups to be prepared. The RGD and similar modifications will be utilized to probe the integrin receptor expression and regulation during foreign body giant cell development. The polysaccharide, i.e. heparin, mannose, etc., modifications are being used to investigate pre-adsorbed blood protein interactions as well as inhibition of the macrophage mannose receptor which is known to regulate the fusion of macrophages to form foreign body giant cells [4].

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