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# Monoclonal Antibodies Detect Monocyte/ Macrophage Activation and Differentiation Antigens and Identify Functionally Distinct Subpopulations of Human Rheumatoid Synovial Tissue Macrophages

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***Monoclonal antibodies (MAbs) to functionally heterogeneous populations of human rheumatoid arthritis (RA) synovial tissue macrophages and lipopolysaccharide (LPS)-activated U937 cells were generated. These MAbs were used to characterize macrophages*** in situ ***in the synovial pannus and to study relative antigen expression on the surface of cells isolated from the synovium and from normal peripheral blood. Monoclonal antibody 3D8, an anti-CD13 MAb, reacts with an antigen expressed on the surface of blood monocytes and is a monocyte activation-related antigen that is upregulated by exposure of monocytes to interferon-gamma (IFN-gamma) and LPS. The expression of the 3D8 antigen increases in parallel with MHC class II antigen expression and also is upregulated in culture as monocytes mature to macrophages. 3D8 antigen is expressed strongly on RA synovial tissue lining cells, which are thought to be composed of macrophages. 8D7 antigen expression, detected by MAb 8D7, increases on blood monocytes on cellular activation with LPS and interferon-gamma, but in contrast to the 3D8 antigen, does not increase with monocyte maturation*** in vitro. ***The 8D7 antigen is expressed differentially on density-defined macrophage subpopulations isolated from RA synovial tissue and is expressed more strongly on macrophages that are nonangiogenic than those that are angiogenic. (Am J Pathol 1991, 138:165–173)***

Rheumatoid arthritis (RA) synovial tissue is an aggressive, hyperplastic, and invasive lesion.[1,2] Proliferation of fibroblastic synovial cells and accumulation of many macrophages and other mononuclear cells occur within this tissue.[2] Previously we showed that macrophages isolated directly from the RA synovium are functionally heterogenous. A density-defined subpopulation of these cells ($F_3$, density 1.042 to 1.062 g/ml) mediated angiogenesis in the *in vivo* rat corneal bioassay, while other subpopulations (such as F2, density 0.998 to 1.042 g/ml) did not.[3] This same angiogenic subpopulation of cells liberated the greatest quantities of chemotactic activity for microvascular endothelial cells and of mononuclear cell factor.[4]

Because tissue sources are not available readily for the isolation of human mononuclear phagocytes, the peripheral blood monocyte often is used to study these cells.[5] Monocytes respond to exogenous and endogenous signals and reprogram their functional state. The reprogramming of mononuclear phagocytes in response to environmental signals is called activation.[6]

To study further the macrophages of the synovial tissue, we raised MAbs using isolated RA synovial macrophages and the lipopolysaccharide (LPS)-activated cell line, U937. In this paper we describe the generation of two MAbs that detect functionally distinct rheumatoid synovial tissue macrophage subpopulations and that detect antigens whose expression is altered by monocyte activation and differentiation.

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## Materials and Methods

### Isolation of Human RA Synovial Tissue Macrophages

Fresh human RA synovial tissues were obtained during total knee or hip surgery from patients with RA who met the diagnostic criteria of the American College of Rheumatology.[7] Synovial tissues were minced and digested in a solution of dispase, collagenase, and DNase, as previously described.[3] The resultant single-cell suspensions were fractionated into density-defined subpopulations by isopyknic centrifugation through continuous preformed Percoll gradients (Pharmacia, Piscataway, NJ) and macrophages were enriched by adherence to fibronectin-coated collagen gels and selective trypsinization (incubation with trypsin: ethylenedinitrilo tetraacetic acid [EDTA] for 5 to 10 minutes).[3,4] Macrophages harvested from the collagen gels by treatment with clostridial collagenase were ≥90% pure, as assessed by $F_c$ receptor-mediated phagocytosis of IgG opsonized sheep red blood cells, esterase staining, and staining with OKM1 (Ortho Diagnostics, Raritan, NJ) and anti-LeuM3 (Becton-Dickinson, Mountain View, CA). After isolation, the macrophages were injected into BALB/c mice for production of MAbs or used for screening (see below).

In addition to using RA synovial macrophages to produce MAbs that might recognize other activation-related myeloid determinants, the human promonocytic cell line U937 was activated with LPS (5 μg/ml) for 20 hours and used as the immunogen.[8]

### Immunization and Fusion

Eight- to twelve-week-old BALB/c mice were immunized by sequential intraperitoneal injection of 0.5 to 2 × $10^7$ macrophages. Three days after the final injection, the mice were killed, their spleens removed, and their splenocytes fused with either the murine myeloma cell line SP 2/0 Ag14 or NS-1 using the technique of Kohler and Milstein.[9]

### Screening of MAbs

#### Control MAbs

For the enzyme-linked immunoassay (ELISA), immunohistochemical staining and radioimmunoassay (RIA) analyses described below, the following commercial control antibodies were used: anti-Leu M3 (anti-CD14, Becton-Dickinson), anti-Leu M5 (anti-CD11c, p150, 95, CR4 receptor, Becton-Dickinson), anti-KP1 (anti-CD68, Dakopatts, Carpinteria, CA), which are MAbs that detect monocytes and macrophages; anti–HLA-Dr (monomorphic) (Becton-Dickinson); and anti-HLA class I (Pelfreez, Brown Deer, WI). Negative controls used were isotype-specific mouse monoclonal immunoglobulin (Coulter, Hialeah, FL) and nonmonocyte reactive hybridoma supernatant produced in our laboratory. All control antibodies and secondary antibodies used in immunoassays were used at saturating concentrations, as determined by antibody titrations (see below).

#### Cellular ELISA

Initial screening of MAbs was performed using a cellular ELISA by a modification of the method described by Stocker et al.[10] Briefly 1.25 × $10^5$ RA synovial tissue macrophages or LPS-activated U937 cells were centrifuged onto poly-L-lysine-coated polystyrene immunoassay plates (Costar, Cambridge, MA). Cells were fixed for 5 to 7 minutes in 0.25% glutaraldehyde and incubated with hybridoma supernatants. After incubation with peroxidase-conjugated goat anti-mouse immunoglobulin (Cappel, Malvern, PA), o-phenylenediamine substrate was added to the ELISA plates and the reaction was read at 414 nm using an ELISA plate reader (Bio-Rad, Richmond, CA). Monoclonal antibodies were selected based on their reactivity with RA macrophage subpopulations or their reactivity with activated U937 cells.

### Determination of Immunoglobulin Class and Subclass

Hybridoma supernatants were concentrated (×10) using Centricon concentrators (10,000 molecular weight cutoff; Amicon, Danvers, MA). Classes and subclasses of MAbs present in the hybridoma supernatants were determined by an Ouchterlony assay using concentrated hybridoma supernatants and the appropriate antibody reagents (Bioproducts for Science, Indianapolis, IN).[11]

### Immunoperoxidase Staining

Immunoperoxidase staining of frozen tissue sections, or methanol-Carnoy's fixed (60% methanol, 30% chloroform, 10% glacial acetic acid) paraffin-embedded tissues was performed using an avidin-biotin technique (Vector Laboratories, Burlingame, CA).[12,13] Slides, air dried for 5 to 7 minutes, were placed in cold acetone for 20 minutes. Endogenous peroxidase activity was quenched by incubating the slides for 30 minutes in 0.3% hydrogen peroxide in methanol. All subsequent incubations were performed for 15 minutes at 37°C in a moist chamber. The tissue sections were pretreated with 50 μl diluted normal horse

serum (135 μl horse serum in 10 ml phosphate-buffered saline 1% bovine serum albumin [PBS-BSA]) and were incubated with MAbs and washed twice. The slides were incubated with a 1:400 dilution of anti-mouse biotinylated antibody in PBS-BSA. Slides were washed twice with PBS, incubated with avidin/biotin reagent, and washed twice with PBS. Slides were stained with diaminobenzidine tetrahydrochloride substrate for 5 minutes at room temperature, rinsed in tap water for 2 minutes, counterstained with Harris' hematoxylin, and dipped in saturated lithium carbonate solution for bluing.

### *Quantitation of Cell-surface Antigens by a Cellular RIA*

Peripheral blood monocytes were isolated using Sepracell-MN (Sepratech, Oklahoma City, OK) according to the manufacturer's instructions. These cells were purified further by plating them on fibronectin-coated collagen gels.[8] The endotoxin concentration of the tissue culture medium was less than 0.05 ng/ml as determined by the Limulus assay (Associates of Cape Cod, Woods Hole, MA). After 2 hours, adherent monocytes were incubated with fresh DMEM + 10% FCS + gentamicin containing either LPS (5 μg/ml) or IFN-gamma (200 units/ml) for 24 hours in a humidified incubator gassed with 5% $CO_2$ and 95% air at 37°C. Previously we used this concentration of LPS to activate human blood monocytes to elaborate angiogenic factors.[8] Interferon (IFN)-gamma has been used at concentrations of 50 to 500 units/ml to stimulate human monocyte and chondrocyte expression of HLA-Dr antigens.[14–18] Incubation times with IFN-gamma varied from 24 to 72 hours for monocyte HLA-Dr expression, and from 5 to 7 days for chondrocyte expression.[14–18] In control experiments, we assayed IFN-gamma concentrations ranging from 50 to 200 units/ml and found 200 units/ml to provide the greatest amount of HLA-Dr stimulation under these conditions.

Monocytes, which were more than 95% pure by esterase staining, were harvested from the collagen gels.[4,8] For time-course studies, adherent monocytes were incubated with DMEM + 10% FCS for up to 9 days. These long-term cultures were fed with fresh medium every 3 or 4 days. After 7 to 9 days of culture, these monocytes acquired morphologic and functional characteristics of macrophages, including an increase in size and development of pseudopodia.

Monocytes, monocyte-derived macrophages, or RA synovial tissue macrophages harvested from the collagen gels were used in an RIA according to the method of Hu.[19] The monocytes or macrophages were suspended at $5 \times 10^6$ cells/ml in PBS/0.3% gelatin (Swine skin gelatin, Sigma Chemical Co., St. Louis, MO)/1% BSA/10% fetal calf serum (FCS). To decrease the binding of murine antibodies to $F_c$ receptors on monocytes, in some cases cells ($1 \times 10^6$) were suspended in 100 μl PBS with 2% FCS, 0.1% $NaN_3$, and 1% heat-inactivated pooled human AB serum for 30 minutes at 4°C. Subsequently cells ($1.4 \times 10^5$) were added to each well of a modified 96-well microtiter plate containing a filter manifold attachment (V and P Scientific Inc., San Diego, CA).[19] The filters were presoaked in the gelatin/BSA/FCS buffer for 30 minutes before use. The monocyte or macrophage suspension was incubated for 1 hour at 37°C with the MAbs. After washing the cells (×3) with PBS containing 0.3% gelatin, 50,000 counts per minute (cpm) of $^{125}$I-labeled goat anti-mouse IgG (ICN Biomedical, Costa Mesa, CA) in 50 μl of buffer containing 0.3% gelatin/0.01 mol/l (molar) PBS/1% BSA were added. After a 30-minute incubation at 37°C, the cells were washed four times with PBS/0.3% gelatin, the filter discs removed, and assayed for radioactivity using a gamma counter. Both primary and secondary antibodies were used at saturating concentrations. All assays were performed in triplicate and the results expressed as the mean ± the standard error of the mean.

### *Statistical Analysis*

Statistical analyses were performed using analysis of variance (ANOVA).[20] *P* values less than 0.05 were considered significant. If there were significant findings using ANOVA, a series of paired *t* tests was conducted to identify differences among groups according to Fisher's protected *t* procedure.[21]

## *Results*

### *Immunization, Fusions, and Immunoglobulin Class and Subclass of Monoclonal Antibodies*

Four individual fusions were performed to select the MAbs studied. Monoclonal antibody 3D8 was derived from the fusion of immunized BALB/c mouse spleen cells and SP 2/0 Ag14 cells. Monoclonal antibody 8D7 was derived from fusion of mouse spleen cells and NS-1 cells. The immunogen used to generate MAb 3D8 was U937 cells stimulated with LPS, while MAb 8D7 was generated from mice immunized with RA macrophages. Monoclonal antibody 3D8 was characterized by reaction with class and subclass antibody reagents as being IgG2a, while MAb 8D7 was an IgG1 antibody.

### In Situ *Localization of Macrophages Identified by the MAbs by Immunohistochemistry*

A summary of the reactivity patterns of the MAbs derived from examination of 10 RA synovial tissues is shown in

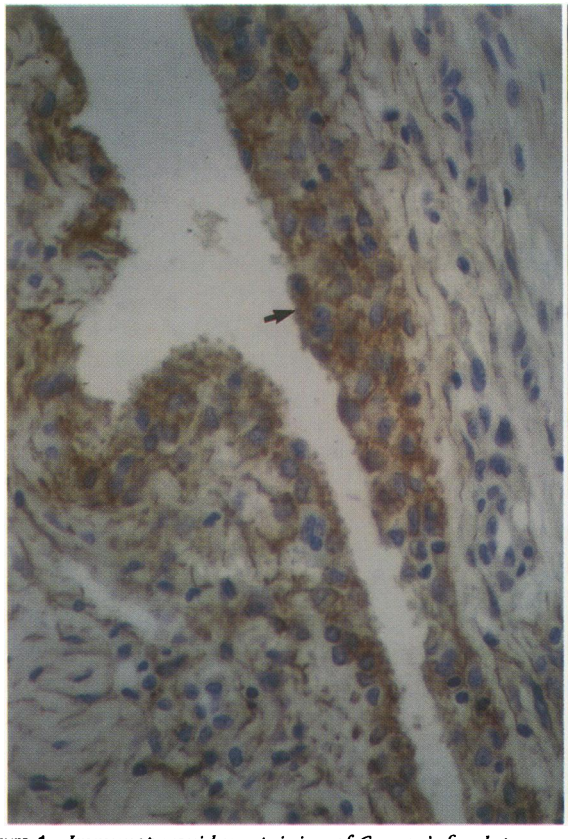

**Figure 1.** *Immunoperoxidase staining of Carnoy's fixed, paraffin-embedded rheumatoid arthritis synovial tissue. Strong staining of the synovial lining layer with MAb 3D8 is shown (arrow) (500×).*

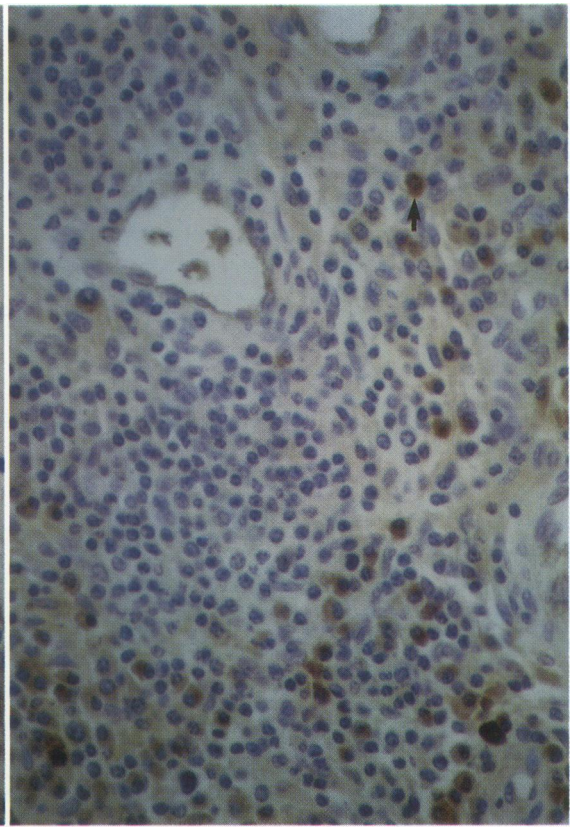

**Figure 2.** *Immunoperoxidase staining of a Carnoy's fixed, paraffin-embedded rheumatoid arthritis synovial tissue section with MAb 8D7 is shown. Strong staining of endothelial cells and macrophages (arrow) within the synovium is present (500×).*

Table 1. While all of the MAbs reacted with macrophages throughout the RA synovial tissue, the synovial lining layer stained with MAb 3D8. Monoclonal antibody 8D7 stained the synovial lining layer in some, but not all of the tissues studied. To confirm the macrophage reactivity of anti-8D7, serial tissue sections were reacted with anti-KP1 (an anti-CD68 MAb). Both MAbs 8D7 and KP1 reacted with cells within the tissues that had macrophage morphology. Less than one half of the tissues contained occasional cells in the lining layer staining positively with anti-LeuM5 (Figures 1 and 2).

**Table 1.** *Monoclonal Antibody (MAb) Reactivity to RA Synovial Tissues as Determined by Immunohistochemistry*

| | MAb designation | | |
|---|---|---|---|
| Tissue Region | 3D8 | 8D7 | anti-LeuM5 |
| Synovial lining layer | + | +† | −‡ |
| Subsynovial layer | | | |
| Macrophages | + | + | + |
| Endothelial cells | −* | + | − |
| Lymphocytes | − | − | − |
| Lymphoid follicles | − | − | − |
| Fibroblasts | −* | −* | − |

* Denotes occasional weak staining in some samples.
† Denotes positive staining in some but not all samples.
‡ Denotes predominently negative staining. In some samples, isolated cells within the synovial lining layer were stained.

## *Quantitative Measurement of the Reactivity of Antibodies with Peripheral Blood Monocytes: Comparison with Synovial Tissue Macrophages*

### *Binding of MAbs to RA Synovial Tissue Macrophages and Normal Blood Monocytes*

Radioimmunoassays were performed using peripheral blood from four healthy volunteers and isolated synovial tissue macrophages from seven patients with RA. Figure 3 shows the relative binding of the MAbs to peripheral blood monocytes. Monoclonal antibody 3D8 binding to resting cells exceeded the binding of anti-HLA-Dr and of other antimyeloid antibodies. Given the caveat of differential binding due to different binding avidities, both MAbs

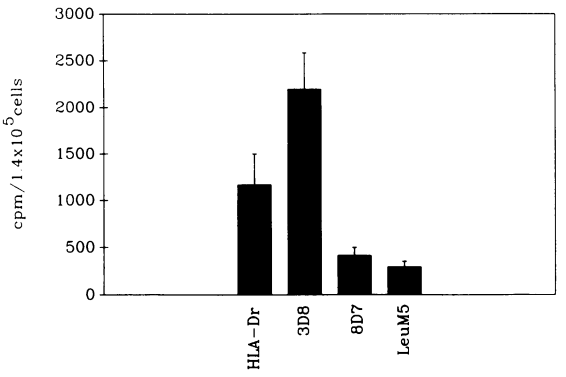


**Figure 3.** *Relative binding of the MAbs to human peripheral blood monocytes from four donors was determined by a cellular radioimmunoassay (see text). Results are expressed above background counts obtained using isotype-specific control antibodies. All assays were performed in triplicate. Results represent the mean ± SE.*

3D8 and 8D7 had a greater degree of binding to resting monocytes than did anti-LeuM5.

Figure 4 shows the binding of the MAbs to $F_3$ RA synovial tissue (angiogenic) macrophages. In contrast to the binding on peripheral blood monocytes, anti HLA-Dr exhibited greater binding to $F_3$ macrophages than did MAb 3D8. Monoclonal antibody 8D7 binding was markedly increased in comparison to its binding on peripheral blood monocytes.

We compared the relative binding of MAb 3D8 and anti-HLA-Dr on monocytes and $F_3$ synovial macrophages (Figure 5). Monoclonal antibody 3D8 binding, like that of HLA-Dr, markedly increased with monocyte activation, both with LPS and IFN-gamma. While the mean anti-HLA-Dr binding on nonactivated monocytes was 1163 ± 291 cpm, the mean binding on LPS-stimulated monocytes was 1652 ± 274 cpm and on interferon-gamma stimulated monocytes was 3091 ± 645 cpm ($P = 0.02$, significant). 3D8 antigen expression was 2193 ± 316 cpm on nonactivated monocytes *versus* 2922 ± 353 cpm ($P = 0.04$, significant) on LPS-activated monocytes, and 3278 ± 222 cpm on interferon-gamma–stimulated monocytes. It is important to note that these determinations were on a per-cell basis and did not distinguish between large cells with a low antigen density and small cells with a high antigen density. On a per-cell basis, MAb 8D7 binding was greater on $F_3$ macrophages (1409 ± 230 cpm) compared to peripheral blood monocytes (414 ± 73 cpm; $P = 0.006$, significant). Both MAbs 3D8 and 8D7 showed greater binding to synovial tissue macrophages than to peripheral blood monocytes. As with MAb 3D8, MAb 8D7 binding (414 ± 73 cpm) was increased on monocyte stimulation with LPS (700 ± 155 cpm; $P = 0.074$, not significant) or IFN-gamma (1405 ± 303 cpm; $P = 0.019$, significant). Anti-LeuM5 binding also increased slightly with monocyte activation and was greater on $F_3$ macrophages compared to peripheral blood monocytes. However the degree of binding never approached that of MAbs 3D8 or 8D7, and the increase was not statistically significant.

## *Determination of Antigen Expression During Monocyte Differentiation*

To determine whether MAb 3D8 and MAb 8D7 binding was altered with cellular maturation, as well as with cellular activation, adherent monocytes were maintained in culture for up to 9 days and MAb binding was measured at various times. After 7 days in culture, the cells underwent characteristic morphologic changes, including an increase in size and development of pseudopodia, acquiring the characteristics of mature macrophages. As shown in Figure 6, MAb 8D7 binding to monocytes was 505 ± 163 cpm at 2 hours in culture and fluctuated slightly over successive days, but was approximately the same by day 9 in culture (664 ± 61 cpm). By contrast, MAb 3D8 binding increased steadily with monocyte maturation *in vitro* (1903 ± 81 cpm at 2 hours in culture, compared to 4527 ± 747 cpm at day 9 in culture). The greatest increase in 3D8 binding occurred at days 7 to 9 of culture, when monocytes acquire the characteristics of macrophages.

## *Determination of Macrophage Antigen Expression on Functionally Distinct RA Macrophage Subpopulations*

To correlate MAb binding with the functional subsets of RA synovial tissue macrophages that we previously defined, we compared binding on $F_3$ (angiogenic) and $F_2$ (nonangiogenic) macrophages. All of the MAbs tested, including anti-LeuM5, bound approximately equally to both cell populations, with the exception of MAb 8D7, which

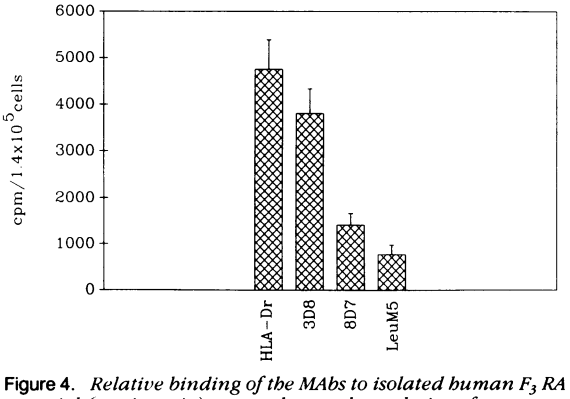


**Figure 4.** *Relative binding of the MAbs to isolated human $F_3$ RA synovial (angiogenic) macrophage subpopulations from seven patients determined by radioimmunoassay. All assays were performed in triplicate. The results represent the mean ± SE.*

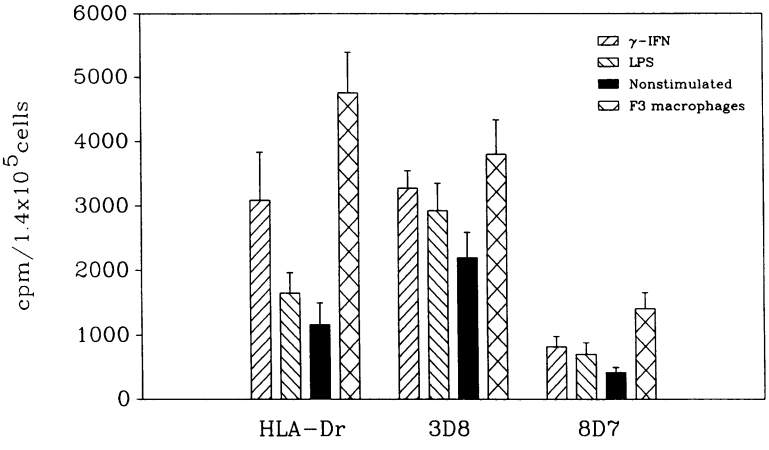


**Figure 5.** *Relative binding of MAbs 3D8, 8D7, and anti-HLA-Dr to peripheral blood monocytes and $F_3$ RA tissue macrophages determined by radioimmunoassay. Monocytes were cultured in the presence and absence of lipopolysaccharide (5 ug/ml) and interferon-gamma (200 u/ml) for 24 hours. Radioimmunoassays were performed simultaneously on blood monocytes and rheumatoid arthritis tissue macrophages. The results represent the mean of determination on monocytes from four normal volunteers and tissue macrophages from seven rheumatoid arthritis patients. All assays were performed in triplicate. The results are represented ± SE. The statistical analysis of this data is described in the text.*

showed significantly greater binding to $F_2$ macrophages. Anti-HLA-Dr expression was virtually identical on the surface of the two subpopulations (data not shown). A comparison of MAb 8D7 binding with another representative anti-macrophage antibody produced in our laboratory, called MAb 10G9, is shown in Figure 7.

## Discussion

Monoclonal antibody 3D8 was raised to LPS-activated U937 cells, while MAb 8D7 was raised to RA synovial macrophages. Fortuitously MAb 3D8 detected an activation-related antigen that is present also on macrophages in the RA synovium. MAb 3D8, which recently was classified as an anti-CD13 MAb,[22] appears to be a monocyte activation-related antigen. Monocyte activation is considered an integral stage in the immunopathogenesis of RA. There are relatively few MAbs that mark monocyte activation antigens. Class II antigens are upregulated on activated monocytes and macrophages, particularly on exposure to IFN-gamma.[23-27] Interleukin-2 receptors were shown to be increased on alveolar macrophages in patients with sarcoidosis and are inducible by lymphokine treatment of normal human lung macrophages, blood monocytes, and monocyte cell lines.[28,29] Anti-A1-3, an antibody that inhibits monocyte procoagulant activity, is reported to react selectively with activated monocytes and macrophages.[30,31] Morganelli et al[32] described a series of MAbs that identify a 155-kd antigen (gp155) on monocytes that is selectively upregulated on stimulation with IFN-gamma and dexamethasone. Mo3e antigen is upregulated with exposure of monocytes to LPS, PMA, or muramyl dipeptide but not with exposure to IFN-gamma and appears to be associated with the monocyte response to the migration-inhibitory factor.[33] To these MAbs that detect antigens upregulated with cellular activation can be added MAb 3D8 reported here.

In general, monocyte activation antigens have been linked to the process of monocyte differentiation. 3D8-antigen expression steadily increases as monocytes mature in culture, with the maximum rate of increase occurring from days 7 to 9 of culture, a time when cultured monocytes acquire the morphologic and functional characteristics of macrophages. Recently it was determined that CD13 is identical to aminopeptidase N, a membrane-bound glycoprotein.[34] There have been limited studies on aminopeptidases as mononuclear phagocyte markers in murine systems, indicating that aminopeptidases are increasingly expressed as bone marrow-derived mononuclear phagocytes mature in culture.[35] In these studies, the particular class of aminopeptidase was not identified. It is likely that in human systems, MAb 3D8 also identifies a myeloid maturation-related antigen.

This maturation-related increase in antigen expression also was reported with some other monocyte-activation antigens. For example, the gp155 antigen described by Morganelli et al,[32] which is expressed on freshly isolated

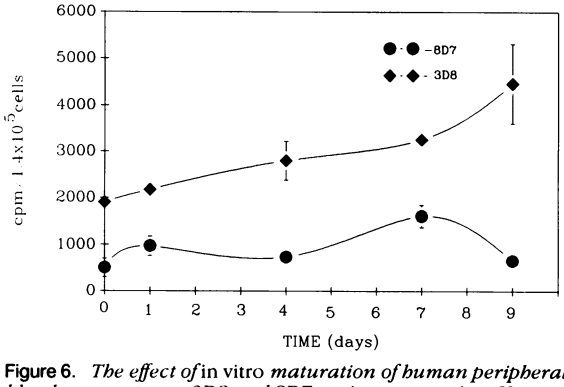


**Figure 6.** *The effect of* in vitro *maturation of human peripheral blood monocytes on 3D8 and 8D7 antigen expression. Human peripheral blood monocytes from three normal volunteers were cultured on fibronectin-coated collagen gels for up to 9 days in RPMI + 10% FCS + gentamicin. Cells were harvested at the indicated time intervals and assayed in a cellular radioimmunoassay. All assays were performed in triplicate. The results represent the mean ± SE.*

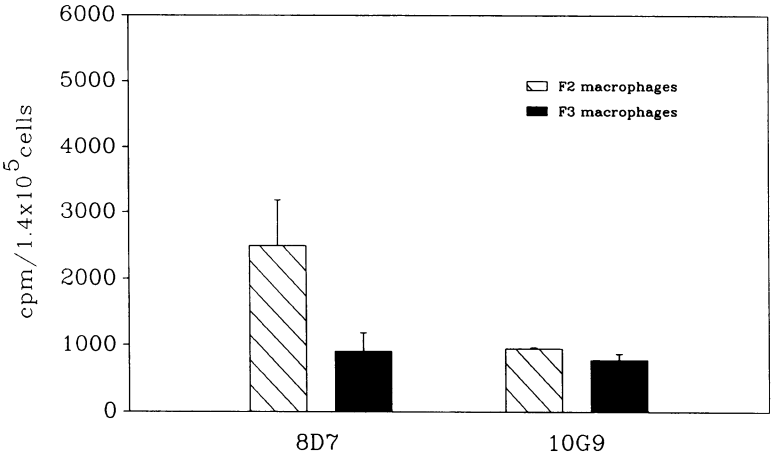


**Figure 7.** *The relative binding of MAbs 8D7 and 10G9 on rheumatoid arthritis synovial macrophages from three patients was determined by radioimmunoassay. In contrast to 10G9 antigen, which is expressed almost equally on both subpopulations, 8D7 antigen is increased on $F_2$ (nonangiogenic) macrophages compared to $F_3$ (angiogenic) macrophages. All assays were performed in triplicate. The results are expressed as the mean ± SE.*

peripheral blood monocytes, is greatly upregulated with 5-day *in vitro* culture of monocytes. Similarly Mo3e antigen expression is increased by incubation of HL60 human myelocytic leukemia cells with PMA.[36] The expression of Mo3e by these stimulated HL60 cells coincides with the development of features of monocyte differentiation (characteristic morphology, nonspecific esterase activity, and respiratory burst activity).[33]

Unlike myeloid antigens that increase with monocyte differentiation, 8D7 antigen expression, although increasing with monocyte activation, does not increase appreciably as blood monocytes mature in culture. Similarly MAb EBM11, an anti-monocyte MAb, appears to detect monocyte maturation independently of activation (class II antigen expression).[37] Hence monocyte activation does not appear to be strictly linked to monocyte differentiation.

Rheumatoid arthritis synovial tissue macrophage phenotype has been examined by others using anti-myeloid MAbs. The RA synovial lining layer was described as being strongly HLA-Dr positive and reacting with the anti-CD14 MAbs 63D3 and FMC-17.[38,39] Burmester et al[40] identified populations of macrophages isolated from the RA synovium that express HLA-Dr, have $F_c$ receptors, and stain with MAbs to different monocyte lineage differentiation antigens (macrophage-1 [anti-CD14], macrophage P-9 [anti-CD14], macrophage P-15 [anti-CD14], and macrophage R-17) to a greater extent than do blood monocytes. Allard et al,[41] and Hogg et al[42] identified abundant cells in the synovial lining layer that bear HLA-Dr, HLA-Dp, and HLA-Dq but fewer cells that bear CD11c, and therefore hypothesized that these cells may represent macrophages in which normal cell-surface markers were downregulated. Other authors also found occasional CD11c-positive staining cells within the lining layer.[43] The sublining layer contained many cells expressing class II antigens, CR4 receptors, and CD14, and may represent macrophages actively involved in antigen presentation.

Firestein et al[14] studied peripheral blood and synovial fluid from patients with RA and found that RA synovial fluid monocytes were 'activated,' with high expression of HLA-Dr and low expression of CD14. In contrast, RA peripheral blood monocytes were 'resting,' with the converse phenotype. HLA-Dr expression on synovial fluid monocytes could not be upregulated by IFN-gamma exposure while CD14 antigens were downregulated by exposure to IFN-gamma. These authors suggested that RA synovial fluid monocytes were previously activated, compared to peripheral blood monocytes, and that IFN-gamma may not be the only signal playing a role in activating RA monocytes.

In contrast to the expression of CD14 antigen, we found that the CD13 antigen, detected using MAb 3D8 (anti-CD13), is expressed strongly on RA synovial lining cells, as well as on some macrophages deep within the synovium. Burmester et al[40] described a 'double configuration' of the RA synovial lining layer, with cells positive for Ia and CD14 antigens located in the immediate surface of the synovium (layer I), separated by a zone of Ia-negative, CD14-negative cells (layer II) from the intensely stained macrophages present in the deeper synovial areas. Using immunohistochemistry, we confirm the report by Emmrich et al[43] that CD13 is present not only in layer I but also on cells in layer II. Anti-8D7 reactivity, when present on the synovial lining layer, also was present in layer I and II. Based on the results of our immunohistochemical staining, we suggest that as monocytes enter the synovial tissue from the blood, they rapidly migrate to the synovial lining layer where they then bear both class II *and* some monocyte/mø activation antigens, such as CD13. At the same time, as others have shown, other monocyte/macrophage antigens, such as CD14, may be downregulated.

With regard to the functionally distinct subpopulations of RA synovial tissue macrophages we reported previously,[3] we find that the 8D7 antigen, unlike the other an-

tigens identified by the MAbs studied, appears to be upregulated on $F_2$ nonangiogenic RA synovial tissue macrophages, compared to $F_3$ angiogenic macrophage subpopulations. This is in contrast to HLA-Dr, which is expressed equally on these two functionally distinct subpopulations. Further studies to determine whether peripheral blood monocytes and synovial fluid monocytes from RA patients bear this antigen are in progress.

We describe two new MAbs that may help distinguish functionally distinct subpopulations of RA macrophages and detect antigens expressed by monocytes during activation and differentiation.

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