# Zymosan Modulates CD44 Isoform Expression in a Murine Model of Inflammation Resembling Rheumatoid Arthritis Synovitis

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***ABSTRACT.*** ***Objective.*** To study local inflammation induced by zymosan in the murine air pouch, considered a model of synovial-like tissue inflammation, we investigated the time-course synthesis of CD44 and tumor necrosis factor-α (TNF-α) mRNA and established a relationship with leukocyte migration into the air pouch and CD44 expression on the leukocyte membrane.

***Methods.*** Leukocytes from the air pouch exudate were collected and counted at 1, 4, 12, 24, 48, and 72 h after zymosan or saline injection. CD44 and TNF-α mRNA were studied by RT-PCR. CD44 variable exon analysis was assessed by Southern blot and CD44 membrane expression by flow cytometry.

***Results.*** Leukocyte accumulation after zymosan injection was significantly higher than in saline injected controls. CD44 standard and variable isoforms including at least variable exons v6 and v9 were highly expressed in leukocytes from the zymosan air pouch exudate. In contrast, only the CD44 mRNA standard isoform was present in leukocytes from saline air pouch. Maximal TNF-α mRNA level was observed at 48 h after zymosan injection, whereas CD44 mRNA was constantly expressed throughout the whole term of the experiment, although variations in leukocyte count and relative formula were observed.

***Conclusion.*** Expression of CD44 variable isoform in leukocytes was specifically induced by zymosan, since none was detected in saline controls. TNF-α mRNA expression and leukocyte count at every time point served as markers for local inflammation. The presence of variable isoforms, including at least exons v6 and v9, consistently expressed throughout the assay suggests that they could play a role in this arthritis-like inflammation induced under zymosan stimulus. (J Rheumatol 2001;28:943–9)

*Key Indexing Terms:*
AIR POUCH ZYMOSAN ARTHRITIS-LIKE INFLAMMATION CD44 ISOFORMS

The air pouch induced by subcutaneous injection of sterile air into the back of a mouse forms a cavity lined with cells that resembles the synovial membrane after 6 days[1]. Zymosan injection into the air pouch induces arthritis-like inflammation in a similar way as it does in joints[2-4]. The zymosan air pouch model has been used to evaluate the efficacy of antiinflammatory drugs as well as the kinetics of production of various inflammatory mediators such as myeloperoxidase, eicosanoids, secretory phospholipase $A_2$, and nitric oxide[3,5,6]. Zymosan or other soluble high molecular weight ß-glucans induce degranulation and respiratory burst of neutrophils, as well as tumor necrosis factor-α (TNF-α) and interleukin 1 (IL-1) and IL-6 production by monocytes and macrophages[7-9].

CD44 is a transmembrane glycoprotein known to mediate binding to extracellular matrix components as well as cell-cell adhesion[10-13]. Murine CD44 molecules are encoded by 20 exons, of which at least 10 can be spliced out or inserted in various combinations into the extracellular portion behind exon 5 to form diverse variable isoforms with distinctive adhesive properties. The standard form of CD44 lacks all 10 alternatively spliced exons and predominates in most cell types[14,15]. CD44 is the major receptor for hyaluronic acid (HA)[16], widely distributed in leukocytes, mediating their binding to high endothelial venules[17-21]. Another important feature of CD44 is the overexpression of this molecule in various inflammatory conditions including rheumatoid arthritis (RA)[22-24]. Upregulation of CD44 in RA synovitis has been considered a requirement for lymphocyte and monocyte infiltration. Moreover, IM7 anti-CD44 monoclonal antibody (Mab) was shown to reduce leukocyte infiltration and tissue edema in experimentally induced murine arthritis[25-27]. It has been proposed that CD44 on lympho-

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cytes interacts with HA on endothelium and participates in preferential homing of activated lymphoblasts to tertiary inflammation sites[21,28]. CD44 has been proposed to mediate signal transduction since either its cross-linking by Mab, or monocyte or macrophage ligation by HA, induces the release of IL-1 and TNF-α[12,29]. Low molecular weight HA induces production of IL-12 and chemokines RANTES, MIP-1ß, and MIP-1α in stimulated but not in resident peritoneal murine macrophages[30]. CD44–HA interaction may thus be required to augment macrophage effector functions besides contributing to migration into inflammatory sites.

Interestingly, in the mouse air pouch model, the lipid A moiety of bacterial lipopolysaccharide has been shown to induce CD44 upregulation in synovial-like tissue lining the cavity[31]. We evaluated zymosan time-course modulation of CD44 mRNA and correlated it with leukocyte migration into the air pouch, together with TNF-α mRNA synthesis and CD44 membrane expression. Our results showed that CD44 is highly expressed in leukocytes of the zymosan air pouch exudate and that differential expression of CD44 isoforms, including at least exons v6 and v9, is present from the earliest time of infiltration, suggesting that these isoforms could play a role in the inflammatory process induced by zymosan stimulus.

## MATERIALS AND METHODS

*Animals*. NIH mice from the Malbran Institute colony (28–32 g weight) were used for all experiments. They were maintained on Cargill pellets and water ad libitum. Animal studies were conducted in accord with NIH Guide for the Care and Use of Laboratory Animals.

*Mouse air pouch*. Air cavities were produced by subcutaneous injection of 5 ml of sterile air into the back. Three days later 3 ml of sterile air were injected into the cavity. Six days after initial air injection, 0.3 ml of sterile saline or 0.3 ml of 1% (w/v) zymosan (Sigma, St. Louis, MO, USA) in saline was injected into the air pouch[6,32]. At 1, 4, 12, 24, 48, and 72 h after injection, mice were killed by cervical dislocation and the exudate harvested from each pouch by washing out with 6 ml of sterile RPMI (Gibco-BRL, New York, NY, USA) without phenol red, containing 3 mM EDTA. After 10 min centrifugation at 1200 rpm at 4°C, the cell pellet was used for further reverse transcription-polymerase chain reaction (RT-PCR) analysis or flow cytometry. The number of leukocytes present in exudates and relative formula were determined in a Coulter counter analyzer. Peripheral blood leukocytes collected from zymosan or saline injected mice at 24 h postinjection, as well as from normal noninjected mice, were obtained from peripheral blood after 30 s incubation in erythrolyse lysing buffer (Serotec, Oxford, England).

*Flow cytometry.* One million cells were stained with either 1 µg of anti-pan-CD44 unlabeled Mab (IM7 clone; PharMingen, San Diego, CA, USA) or with normal rat IgG (isotype control; Santa Cruz Biotechnology, Santa Cruz, CA, USA) in 100 µl phosphate buffered saline (PBS) for 30 min at 4°C and then washed with PBS. FITC labeled $F(ab)'_2$ goat anti-rat immunoglobulin (Serotec) was added for an additional 30 min. Cells were washed again and analyzed using a FACScan (Beckton Dickinson, San José, CA, USA). A total of 10,000 cells per tube was analyzed and data acquired in list mode were processed using WinMDI software (Scripps Institute, La Jolla, CA, USA).

*RNA isolation and purification*. Total RNA was purified with trizol reagent (Gibco-BRL) according to the manufacturer's protocol. Briefly, cell pellets were homogenized in trizol solution and phase separation with chloroform was performed. RNA was precipitated from the aqueous phase by mixing with isopropanol, the precipitate was washed with 75% ethanol and then suspended in DEPC treated water. Optical density (OD) at 260 nm was measured to determine RNA concentration.

*cDNA synthesis*. cDNA synthesis of mRNA was carried out by reverse transcription using $oligo(dT)_{15}$ primers. The reaction mixture contained $oligo(dT)_{15}$ primers, dNTPs, 1 µg RNA sample in 5 µl DEPC treated water; after heating at 70°C for 10 min and cooling on ice, MMLV-RT buffer, RNAsin, and MMLV-RT (Promega, Madison, WI, USA) were added. Samples were first incubated at 37°C for 90 min and then at 95°C for 5 min to inactivate reverse transcriptase.

*PCR amplification*. cDNA samples were then amplified in a PCR reaction mixture consisting of sense and antisense oligonucleotides, DEPC treated water, 10× Taq buffer, dNTPs, and Taq DNA polymerase (Promega). All reaction mixtures were overlaid with mineral oil, then subjected to different cycles of amplification and to a final extension of 72°C for 10 min using a programmed thermal cycler (Perkin Elmer). CD44 isoform amplification was performed as described[33], using CD44 S1 and CD44 2A primers, which are complementary to constant exons 5 and 6, respectively. Cycles were as follows: 94°C for 20 s, 55°C for 60 s, 72°C for 120 s, with a total of 35 cycles. For TNF-α 1s and TNF-α 2A primers: 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, with a total of 35 cycles. For ß-actin A and ß-actin B: 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min, with a total of 25 cycles. PCR samples were analyzed by electrophoresis on 2% TAE agarose gels containing ethidium bromide at 75 V for 1.5 h. Gel images were scanned and densitometric analysis was performed using Kodak 1D3.0 USB software.

*Southern blot analysis*. CD44 variable exon analysis was performed by Southern blot hybridization and nonradioactive detection according to the manufacturer's instructions (Boehringer Mannheim, Indianapolis, IN, USA). CD44 PCR products separated on 2% agarose gels were denatured, neutralized, and transferred to nylon membranes (Sigma) by capillary transfer in 20× SSC (3 M NaCl, 0.3 M sodium citrate) overnight. After UV cross-linking of the transferred membrane and prehybridization in prehybridization solution [5× SSC, 1% blocking reagent for nucleic acid hybridization, 0.1% laurylsarcosine, 0.2% sodium dodecyl sulfate (SDS)] at 42ºC for 1 h, samples were hybridized at 42ºC overnight with 2 different digoxigenin labeled probes specific for variable exons v6 and v9. Membranes were then washed at 42ºC with 2× and 0.5× washing solution (2× and 0.5× SSC, respectively, and 0.1% SDS) and equilibrated with Genius Buffer (100 mM Tris-HCl, 150 mM NaCl) at room temperature. After blocking in 0.5% gelatin and 1% milk to avoid nonspecific protein interactions, they were incubated 30 min at room temperature with alkaline phosphatase labeled anti-digoxigenin Fab fragment (Boehringer Mannheim). After washing in Genius Buffer and enzyme activation (100 mM Tris-HCl, pH 9.5, and 100 mM NaCl), CSPD substrate (Boehringer Mannheim) was added and incubated for 5 min. The membrane was exposed to Agfa X-ray film for 15 min and developed.

*Statistical analysis*. To compare values for time-course data of zymosan injected mice, one-way analysis of variance was used followed by Tukey's test. Cell count comparison with saline controls was analyzed by Student's t test.

## RESULTS

*Leukocyte infiltration in the mouse air pouch exudate*. Zymosan injected mice exhibited a significant time dependent increase in total leukocyte count that peaked at 12 h. Total count remained as high as $10^7$ cells at 72 h postinjection. In contrast, total leukocyte count of saline injected mice remained lower than $10^6$ throughout the whole term of the experiment and exhibited no variations (Figure 1).

The differential leukocyte count kinetics revealed a time-

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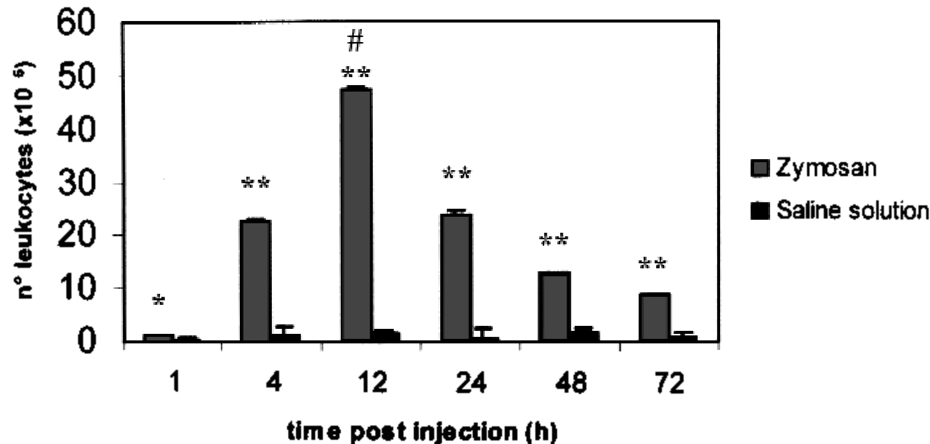


*Figure 1.* Accumulation of inflammatory cells in air pouches at different time points after saline or zymosan injection. Vertical lines show SD (n = 3). Mean values for zymosan injected air pouches were significantly higher than for saline injected air pouches for all time points. *p < 0.05; **p < 0.01. Total count peaked at 12 h (#p < 0.01).

course decrease in granulocyte rate, while the mononuclear rate increased up to 72 h. Maximal total cell count at 12 h of zymosan injected mice mainly consisted of granulocytes (68%), while at 72 h mononuclear cells predominated (56%) (Figure 2). The fraction of monocytes increased slightly from 15% at 24 h to a peak of 22% at 48 h and later decreased to 18% at 72 h.

*Time-course expression of TNF-α mRNA.* As shown by RT-PCR, leukocytes from zymosan injected air pouch exudate exhibited a time dependent course of TNF-α mRNA expression at 12, 24, 48, and 72 h, with a marked increase at 48 h compared with ß-actin controls in a semiquantitative analysis (Figure 3). Leukocytes from saline injected mice at the same time points exhibited substantially lower TNF-α mRNA expression, which in some cases proved undetectable (data not shown).

*Time-course expression of CD44 mRNA.* RT-PCR revealed CD44 mRNA expression of the standard isoform and variable isoforms of higher size in leukocytes from zymosan injected mice, evident at 12, 24, 48, and 72 h. Highly expressed variable isoforms were only seen on leukocytes from zymosan injected mice, and were not present in saline controls or in leukocytes from peripheral blood of mice injected with either zymosan or saline or normal noninjected mice (Figure 4).

*Expression of CD44 variable exons v6 and v9.* Southern blotting of CD44 PCR samples from zymosan injected animals disclosed the presence of variable exons v6 and v9 at 12, 24, 48, and 72 h, while leukocytes from peripheral blood of normal noninjected mice showed no such expression (Figure 5).

*CD44 membrane expression on zymosan air pouch leuko-*

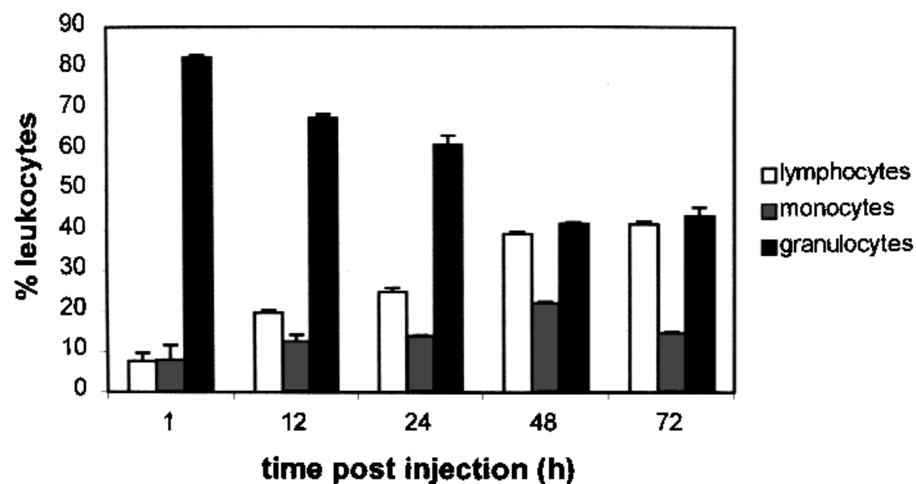


*Figure 2.* Kinetics of differential leukocyte counts in the mouse air pouch after zymosan injection. Bars represent mean values; vertical lines show SD (n = 3).

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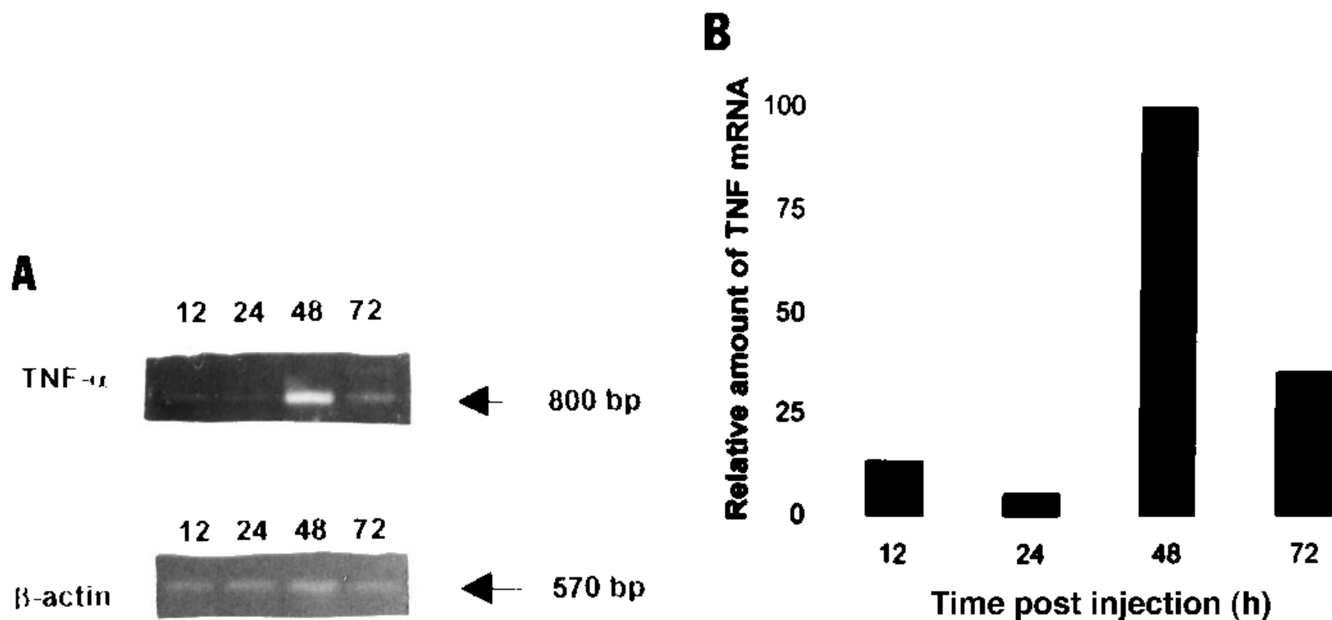


*Figure 3*. A. Time-course expression of TNF-α mRNA performed by RT-PCR at 12, 24, 48, and 72 h in leukocytes from the exudate of zymosan injected air pouches. Sample normalization for equivalent cDNA input was performed using PCR for the housekeeping gene ß-actin to control for variance in initial mRNA amount and reverse transcription efficiency. B. Scanning densitometric analysis of TNF mRNA levels. Values (as relative units) were assigned as a percentage of the most intense band. This is a representative example of 3 similar experiments.

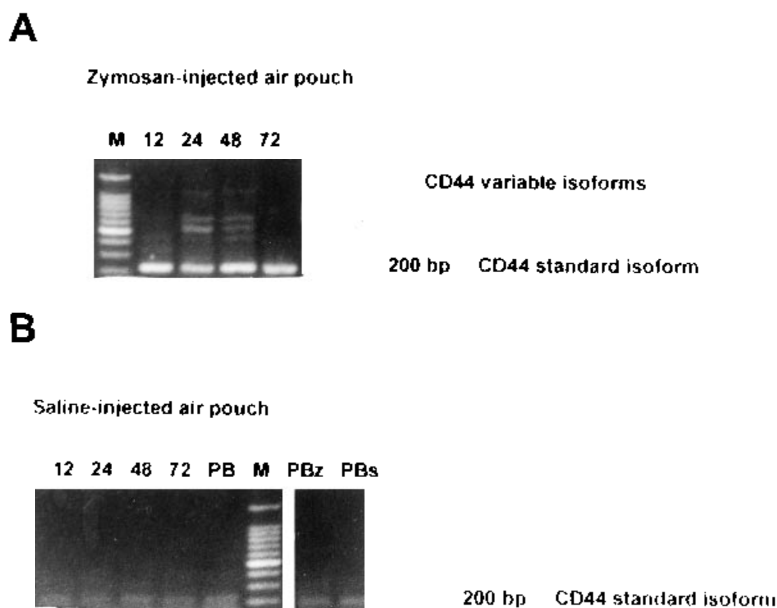


*Figure 4*. RT-PCR analysis of CD44 isoform expression in leukocytes from mouse air pouch exudate after 12, 24, 48, and 72 h of either zymosan or saline injection. The same analysis was performed in peripheral blood leukocytes of normal noninjected mice (PB) and peripheral blood leukocytes of zymosan (PBz) or saline injected mice (PBs). M: DNA ladder 100 bp. This example is representative of 3 experiments.

*cytes*. CD44 expression on the leukocyte membrane was observed by flow cytometry. Leukocytes from zymosan injected air pouch exudate exhibited a high proportion of CD44 positive cells, as determined by FITC staining with anti-pan-CD44 IM7 Mab, at both 24 h (71%) and 72 h (74%) (Figure 6). FACS analysis with leukocytes from saline injected animals also showed CD44 expression with the same Mab (data not shown).

## DISCUSSION

The air pouch model has been considered an ideal tool for investigating the process of inflammatory responses in

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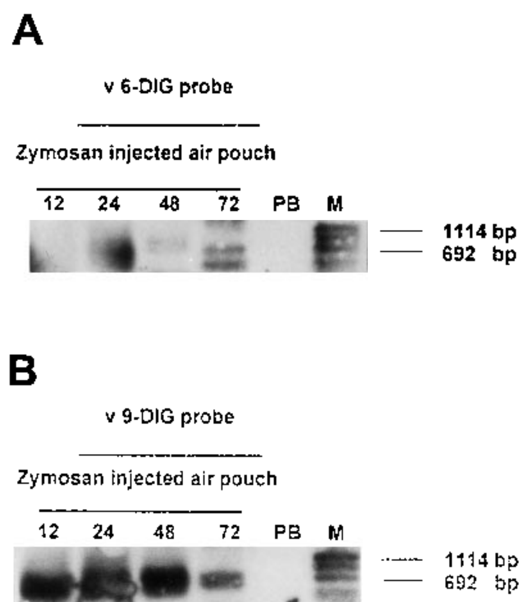


*Figure 5.* Southern blotting of CD44 PCR leukocyte samples obtained from air pouches at 12, 24, 48, and 72 h after zymosan injection. Peripheral blood leukocytes from normal noninjected mice (PB) were used as controls. Panel A: samples hybridized with digoxigenin labeled exon v6-specific probe. Panel B: samples hybridized with digoxigenin labeled v9-specific probe. This is a representative example of 3 similar experiments. M: digoxigenin labeled DNA molecular weight marker.

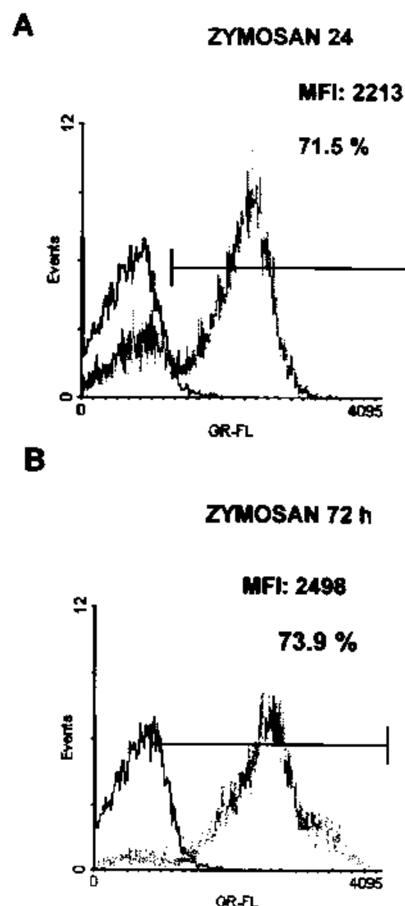


*Figure 6.* FACS analysis of CD44 expression in leukocytes from the mouse air pouch exudate after 24 h (A) and 72 h (B) of zymosan injection (shaded lines). Black lines: isotype control. GR-FL: green fluorescence intensity.

synovium-like tissue such as that of RA[1]. In the rat air pouch, it has been shown that zymosan is phagocytosed and the complement alternative pathway activated; as well, it induces the production of cytokines and various inflammatory mediators such as eicosanoids, nitric oxide, and enzymes such as phospholipase $A_2$ ($PLA_2$). It has been shown that zymosan in rat air pouch induces an exudate containing mainly neutrophils at 4, 8, and 24 h. Maximal granulocyte influx was observed at 8 h, concomitant with the highest level of various critical inflammatory events such as $PLA_2$ activity, inducible NO synthase induction, and NO production[6,32]. In the mouse air pouch injected with lipid A, neutrophils in the lining layer of the pouch peaked at 24 h, while mononuclear cell infiltration did so at 72 h[31]. We also found a predominance of neutrophils at 24 h in the exudate of zymosan injected mouse air pouch, with maximal influx at 12 h, while at 72 h lymphocytes and monocytes were the predominant cells.

In our study we obtained time-course data on the production of TNF-α mRNA by leukocytes of the experimental zymosan-air pouch model and compared these findings with the time-course of CD44 mRNA expression as well as membrane CD44 expression at 24 and 72 h. The marked increase in TNF-α mRNA at 48 h suggests a major contribution of mononuclear cells. In contrast, expression of high CD44 mRNA and membrane CD44 levels throughout the whole term of the assay indicated that both granulocytes and mononuclear cells acquired and maintained a high CD44 expression from the time they migrated into the air pouch exudate, although its relative percentage varied at successive stages during the inflammatory process.

TNF-α has been shown to induce an increase in CD44 expression and to enable CD44 to bind HA on peripheral blood monocytes. It has been also shown that TNF-α plays a leading role in the modulation of CD44 binding to hyaluronan regulated by cytokines, since an anti-TNF-α antibody blocked IL-1α, IL-1ß, IL-2, IL-15, and granulocyte macrophage-colony stimulating factor induced HA binding capacity of CD44 in monocytes. It was found that maximal capacity of CD44 to bind HA was induced by TNF-α in monocytes after 72 h of *in vitro* culture[34]. In our study, TNF-α mRNA was used as a marker for local inflammation and was compared to the time-course of CD44 mRNA expression. Nevertheless, TNF-α increase at 48 h suggests a contribution to further CD44 upregulation and HA binding induction, although this hypothesis requires confirmation.

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Results from our study have shown a high expression of CD44 in the leukocyte exudate of the zymosan injected air pouch, both at 24 h when neutrophils were the most abundant cells and at 72 h, when lymphocytes and monocytes were the major cell fraction. CD44 upregulation has been observed in synovial tissue of patients with RA and more recently in the experimental mouse air pouch model[24,31]. In this last case, CD44 upregulation was identified by immunohistochemical staining of the air pouch lining tissue after injection of lipid A. It has been suggested that high CD44 expression in leukocytes in RA synovial fluid results from selective migration of leukocytes highly expressing CD44 rather than a selective environment provided by the exudate present in synovial fluid[27].

Our findings reveal that there was not only a high expression of standard CD44 but also a differential expression of alternatively spliced isoforms induced by zymosan stimulus, since they were not present in leukocytes from saline injected mouse air pouch. These CD44 isoforms were absent in peripheral blood leukocytes of zymosan injected mice, suggesting that they were induced at the inflammatory site. CD44 isoforms included at least variable exons v6 and v9, both absent in peripheral blood leukocytes. This finding is related to the knowledge that standard CD44 as well as CD44 variable isoform expression was upregulated in RA compared with osteoarthritis synovial cells[35]. Levesque and Haynes have described the presence of v6 and v9 isoforms *in vivo* on tissue macrophages in granulomatous inflammatory infiltrates[36] and in RA in synovial lining cells as well as in scattered synovial tissue macrophages[24,35], and they suggested that CD44 variable isoform expression is associated with *in vivo* differentiation of monocytes to tissue macrophages in inflammatory sites. In addition, Weiss, *et al* observed the presence of activated monocytes expressing variable exons v3, v4, v5, v6, and v9 in cryosections of skin samples from patients with allergic contact dermatitis but not in normal skin[37]. Although we made no attempt to differentiate CD44 variable isoform expression among leukocyte types, our results are in agreement with the concept of variable isoform expression at inflammatory sites, since we found v6 and v9 mRNA from 4 to 72 h post-injury in leukocytes of an *in vivo* model of arthritis-like inflammation.

Using an *in vivo* model of inflammation, the zymosan injected mouse air pouch, we were able to observe high CD44 expression and persistence in leukocytes transmigrating into the air cavity, and to obtain evidence of zymosan-specific induction of CD44 variable isoform expression.

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## REFERENCES

1. Edwards JCW, Sedgwick AD, Willoughby DA. The formation of a structure with the features of synovial lining by subcutaneous injection of air: *in vivo* tissue culture system. J Pathol 1981;134:147-56.
2. Gegout P, Gillet P, Chevrier D, Guingamp C, Terlain B, Netter P. Characterization of zymosan-induced arthritis in the rat: effects on joint inflammation and cartilage metabolism. Life Sci 1994; 55:321-6.
3. Nickerson Nutter CL, Medvedeff ED. The effect of leukotriene synthesis inhibitors in models of acute and chronic inflammation. Arthritis Rheum 1996;39:515-21.
4. Rocha FA, Aragão AG Jr, Oliveira RC, Pompeu MM, Vale MR, Ribeiro RA. Periarthritis promotes gait disturbance in zymosan-induced arthritis in rats. Inflamm Res 1999;48:485-90.
5. Perretti M, Flower RJ. Modulation of IL-1 induced neutrophil migration by dexamethasone and lipocortin 1. J Immunol 1993;148:808-14.
6. Payá M, Terencio MC, Ferrándiz ML, Alcaraz MJ. Involvement of secretory phospholipase $A_2$ activity in the zymosan rat air pouch model of inflammation. Br J Pharmacol 1996;117:1773-9.
7. Doita ML, Rasmussen R, Seljelid R, Lipsky PE. Effect of soluble aminated ß-1,3-D-polyglucose on human monocytes: stimulation of cytokine and prostaglandin $E_2$ production but not antigen presenting functions. J Leukoc Biol 1991;49:342-51.
8. Abel G, Czop JK. Stimulation of human monocyte beta-glucan receptors by glucan particles induces production of TNF-alpha and IL-1 beta. Int J Immunopharmacol 1992;14:1363-73.
9. Ljungman AG, Leanderson P, Tagesson C. (1-3)-ß-D-glucan stimulates nitric oxide generation and cytokine mRNA expression in macrophages. Environm Toxicol Pharmacol 1998;5:273-6.
10. Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B. CD44 is the principal cell surface receptor for hyaluronate. Cell 1990;61:1303-13.
11. Miyake K, Underhill CB, Lesley J, Kincade PW. Hyaluronate can function as cell adhesion molecule and CD44 participates in hyaluronate recognition. J Exp Med 1990;172:69-75.
12. Webb DS, Shimizu Y, Van Seventer GA, Shaw S, Gerrard TL. LFA-3, CD44 and CD45: Physiologic triggers of human monocyte TNF and IL-1 release. Science 1990;249:1295-7.
13. Lesley J, Hyman R, Kincade PW. CD44 and its interactions with extracellular matrix. Adv Immunol 1993;54:271-335.
14. Screaton G, Bell M, Jackson D, Cornelis F, Gerth U, Bell J. Genomic structure of DNA encoding the lymphocyte homing receptor CD44 reveals at least 12 alternatively spliced exons. Prot Nat Acad Sci USA 1992;89:12160-4.
15. Jackson D, Buckley J, Bell J. Multiple variants of the human lymphocyte homing receptor CD44 generated by insertions at a single site in the extracellular domain. J Biol Chem 1992; 267:4732-9.
16. Peach RJ, Hollenbaugh D, Stamenkovic I, Aruffo A. Identification of hyaluronic acid binding sites in the extracellular domain of CD44. J Cell Biol 1993;122:257-64.
17. Denning SM, Le PT, Singer KH, Haynes BF. Antibodies against the CD44 p80 lymphocyte homing receptor molecule augment human peripheral blood T-cell activation. J Immunol 1990;144:7-15.
18. Haynes BF, Telen MJ, Hale LP, Denning SM. CD44: a molecule involved in leukocyte adherence and T-cell activation. Immunol Today 1989;10:423-8.
19. Huet S, Groux H, Caillou B, Valentin H, Prieur AM, Bernard A. CD44 contributes to T cell activation. J Immunol 1989;143:798-801.
20. Stamenkovic I, Amiot M, Pesando JM, Seed B. A lymphocyte molecule implicated in lymph node homing is a member of the cartilage link protein family. Cell 1989;56:1057-62.
21. Siegelman MH, DeGrendele HC, Estess P. Activation of CD44 and

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hyaluronan in immunological systems. J Leukoc Biol 1999; 66:315-21.
22. Fukazawa H, Yoshida K, Ichinohasama R, et al. Expression of the Hermes-1 (CD44) and ICAM-1 (CD54) molecule on the surface of thyroid cells from patients with Graves' disease. Thyroid 1993;3:285-9.
23. Svenningson A, Hannsson GK, Andersen O. Adhesion molecule expression on cerebrospinal fluid T lymphocytes: evidence of common recruitment mechanisms in multiple sclerosis, aseptic meningitis and normal controls. Ann Neurol 1993;34:155-61.
24. Haynes BF, Hale LP, Patton KL, Martin ME, McCallum RM. Measurement of an adhesion molecule as an indicator of inflammatory disease activity. Upregulation of the receptor for hyaluronate (CD44) in rheumatoid arthritis. Arthritis Rheum 1991;34:1434-43.
25. Mikecz K, Brennan FR, Kim JH, Glant TT. Anti-CD44 treatment abrogates tissue oedema and leukocyte infiltration in murine arthritis. Nature Med 1995;1:458-63.
26. Vendrengh M, Holmdahl R, Tarkowski A. Administration of antibodies to hyaluronan receptor (CD44) delays the start and ameliorates the severity of collagen II arthritis. Scand J Immunol 1995;42:353-8.
27. Brennan FR, Mikecz K, Glant TT, Jobanputra P, Pinder S, Nuki G. CD44 expression by leukocytes in rheumatoid arthritis and modulation by specific antibody: implications for lymphocyte adhesion to endothelial cells and synoviocytes *in vitro*. Scand J Immunol 1997;45:213-20.
28. Mohamadzadeh M, DeGrendele H, Arizpe H, Estess P, Siegelman M. Proinflammatory stimuli regulate endothelial hyaluronan expression and CD44/HA dependent primary adhesion. J Clin Invest 1998;101:97-108.
29. Noble PW, Lake FR, Henson PM, Riches DW. Hyaluronate activation of CD44 induces insulin-like growth factor-1 expression by a tumor necrosis factor-alpha-dependent mechanism in murine macrophages. J Clin Invest 1993;91:2368-77.
30. Hodge Dufour J, Noble PW, Horton MR, et al Induction of IL-12 and chemokines by hyaluronan requires adhesion-dependent priming of resident but not elicited macrophages. J Immunol 1997;159:2492-500.
31. Matsukura Y, Takagi T, Okamoto R, Koshino T. Upregulation of CD44 in the inflamed mouse air pouch injected with synthetic lipid A. J Rheumatol 1998;25:539-45.
32. Payá M, García Pastor P, Coloma J, Alcaraz MJ. Nitric-oxide synthase and cyclo-oxygenase pathways in the inflammatory response, induced by zymosan in the rat air pouch. Br J Pharmacol 1997;120:1445-52.
33. Sánchez Lockhart M, Gravisaco MJ, Mongini C, Waldner C, Alvarez E, Hajos S. Alternative exon-specific PCR method for the analysis of human CD44 isoform expression. Oncol Rep 1999;6:219-24.
34. Levesque MC, Haynes BF. Cytokine induction of the ability of human monocyte CD44 to bind hyaluronan is mediated primarily by TNF-$\alpha$ and is inhibited by IL-4 and IL-13. J Immunol 1997;159:6184-94.
35. Hale LP, Haynes BF, McCachren SS. Expression of CD44 variants in human inflammatory synovitis. J Clin Immunol 1995;15:300-11.
36. Levesque MC, Haynes BF. In vitro culture of human peripheral blood monocytes induces hyaluronan binding and upregulates monocyte variant CD44 isoform expression. J Immunol 1996;156:1557-65.
37. Weiss JM, Renkl AC, Ahrens T, et al. Activation dependent modulation of hyaluronate-receptor and of hyaluronate avidity by human monocytes. J Invest Dermatol 1998;111:227-32.

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