Influence of Minor Thermal Injury on Expression of Complement Receptor CR3 on Human Neutrophils

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Thermal injury is well known to inhibit functions of the circulating neutrophil related to its role in host defense against infection, but the mechanism(s) of this phenomenon are not fully understood. To gain further clues to these mechanisms, the authors have studied patients with thermal injury in terms of altered expression of neutrophil cell membrane receptors for the opsonic complement-derived ligand C3bi—complement receptor Type 3, or CR3. CR3 expression was selected for study because an increase in the number of receptors on the cell surface can be stimulated by products of complement activation known to accumulate after thermal injury and because of the role of CR3 in phagocytic and adherence functions of the neutrophil. Expression of CR3 was monitored semiquantitatively by flow cytometry with the use of a murine monoclonal antibody (OKM1) specific for an antigen (CD11) associated with this receptor. Patients evaluated were limited in this study to those with minor degrees of thermal injury (second-degree burn involving <20% of total body surface area) so that possible confounding effects of major injury and its complications could be eliminated. It was observed that patient neutrophil CR3 becomes significantly up-regulated during the first week, as early as 1 day after injury. The maximum level of expression of CR3 averaged >150% (range, 70–314%) of the respective minimum level observed for each patient. The minimum levels of expression of CR3 on patient neutrophils, reached 11–37 days after injury for 7 of 8 patients, were comparable to the level of expression of CR3 on unstimulated control neutrophils. Such temporal up-regulation of patient neutrophil CR3 suggests the early generation of stimuli of CR3 mobilization in response to thermal injury. Increased numbers of CR3 on patient neutrophils may augment microbicidal function and enhance or inhibit delivery of cells to the burn site. (Am J Pathol 1986, 125:563–570)

THERMAL INJURY is well known to alter multiple activities of the polymorphonuclear neutrophil related to its role in host defense against infection. Altered activities described to date include chemotaxis, phagocytosis, secretion, and respiration (reviewed by Till and Ward[1]) The mechanisms that produce these changes, however, remain mostly unknown. With novel reagents now available it has become possible to further define qualities of the neutrophil which are altered after thermal injury for obtaining additional clues to these mechanisms. Such reagents include monoclonal antibodies that react with cell membrane receptors for various products of catabolism of the third component of the complement (C3).

Complement receptor Type 3 (CR3) is one of at least four receptor populations identified on cells of various types that bind one or more peptides derived from C3.[2–5] CR3 expressed on human neutrophils, monocytes, some macrophages, and large granular lymphocytes apparently binds C3bi exclusively.[6,7] The receptor is a heterodimeric glycoprotein consisting of an unique larger alpha chain and a smaller beta chain. The beta chain of CR3 is shared with at least two other leukocyte surface antigens having unique alpha chains, LFA-1 and p150,95.[8] Monoclonal antibodies which have been described to react with antigenic determinants on human CR3 include anti-Mac-1,[3] anti-Mol,[9] MN-41,[10] and OKM1, OKM9, and OKM10.[11]

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Application of anti-CR3 monoclonal antibodies to studies of the human neutrophil has provided evidence that receptor number can be increased by exposure of cells *in vitro* to a variety of stimuli, including *N*-formyl-methionyl-leucyl-phenylalanine,[12,13] Raji cell supernatant,[12] phorbol myristate acetate,[14] calcium ionophore A23187,[13] and C5a des Arg.[15] An increase in expression of neutrophil CR3 *in vivo* has been described for cells from patients undergoing hemodialysis. Such up-regulation of CR3 has been found to involve mobilization of receptors from an intracellular pool associated with specific/secondary cytoplasmic granules of the cell.[13,14] Thus, an increased number of CR3 on the surface of neutrophils *ex vivo* reflects preexposure of the cells to stimuli of secretory function.

In this report we describe the influence of thermal injury on the expression of CR3 on circulating patient neutrophils. Our study has involved the use of monoclonal anti-CR3 antibody, OKM1, and flow cytometry to quantify binding of the antibody. Results obtained demonstrate that neutrophils from patients with minor thermal injury consistently bind an increased amount of OKM1 antibody as early as 1 day after injury, that binding of the antibody can rise to still higher levels over the week following injury, and that normalization of binding occurs during the second week or later after injury. We propose that such modulation of patient neutrophil CR3 may reflect exposure of the cells to complement-derived secretagogues known to be produced in response to thermal injury.

Materials and Methods

Reagents

Murine anti-CR3 monoclonal antibody, OKM1, and fluorescein-conjugated goat anti-mouse immunoglobulin were purchased from Ortho Diagnostic Systems (Westwood, Mass). OKM10 monoclonal antibody was kindly provided by Dr. Gideon Goldstein of Ortho Pharmaceuticals (Raritan, NJ). Phosphate-buffered saline (PBS), bovine serum albumin (BSA), and sodium azide were purchased from Sigma Chemical Co., St. Louis, Mo; *N*-formyl-methionyl-leucyl-phenylalanine (f-Met-Leu-Phe) from Calbiochem, San Diego, Calif; and 10% paraformaldehyde from Polyscience (Warrington, PA).

Control and Study Populations

Blood specimens were obtained from control donors and patients with thermal injury with the approval of the Institutional Review Board of St. Paul-Ramsey Medical Center and the Committee on the Use of Human Subjects in Research of the University of Minnesota. Control donors were office and laboratory personnel. They included 12 women and 5 men of ages ranging from 24 to 40. Eight patients were studied. Table 1 provides information on sex, age, severity of injury, and infection for each of the patients studied. Percent burn injury is given in terms of total body surface area. One patient differed from the others in terms of having experienced considerable inhalation injury and sepsis. Her carboxyhemoglobin level on admission was 44%. She required ventilatory support for the first 72 hours after admission and supplemental oxygen for an additional 2 weeks thereafter. Pulmonary sepsis was diagnosed clinically; urine, eschar, and sputum were culture-positive for *Escherichia coli* and yeast, methicillin-resistant staphylococcus, and lactobacillus, respectively, during a 2-week period beginning 3 days after injury.

Preparation of Leukocytes

Blood specimens were collected in liquid potassium ethylenediamine-tetraacetate (EDTA) vacutainer tubes and cooled immediately to 4 C. Cell counts were performed with an Ortho ELT-8 counter. Cell differentials were determined by microscopic examination of 200 leukocytes from a whole blood smear stained with Wright's stain. All subsequent processing of the cells was carried out at 4 C unless otherwise specified. The whole

Table 1—Description of Patients Studied

Patient	Sex	Age	% Burn injury*	% 2nd degree	% 3rd degree	Inhalation Injury	Sepsis
1	F	23	8	8	0	–	–
2	F	21	15	15	0	–	–
3	M	25	17	17	0	–	–
4	M	19	20	20	0	–	–
5	M	49	15	15	0	–	–
6	M	21	8	8	0	–	–
7	M	51	17	17	0	–	–
8	F	67	8	4	4	+	+

* Given in terms of total body surface area.

blood specimens were diluted with PBS–1% BSA–0.2% sodium azide (PBS-BSA-azide) to adjust the concentration of segmented and band form neutrophils to 5 × 10^6/ml. To 100 µl aliquots of the diluted cells were added 5-µl volumes of primary antibody diluted in PBS-BSA-azide to provide final concentrations of 950 pg/ml. (This concentration of OKM1 antibody was chosen on the basis of results of experiments to determine the amount of primary antibody required to saturate CR3 expressed on nonstimulated control neutrophils and cells preexposed to F-Met-Leu-Phe at a concentration of 10^{-5} M). The cells were then incubated for 30 minutes and washed once with PBS-BSA-azide. Next, erythrocytes were removed by suspension of the cells in lysing medium (0.15 M ammonium chloride, 0.01 M sodium bicarbonate, 0.01 M EDTA, pH 7.4). The leukocytes were washed once again in PBS-BSA-azide and the sedimented leukocytes suspended in 20 µl of autologous plasma plus 100 µl of titered fluorescein-conjugated secondary antibody. After a 30-minute incubation period, the cells were washed three times with PBS-BSA-azide, suspended in PBS containing 1% paraformaldehyde, pH 7.0, and stored in the dark until analysis by flow cytometry.

Flow Cytometry

Flow cytometric analysis of stained leukocytes involved use of a Cytofluorograf 50H and a 2150 computer (Ortho Diagnostic Systems). The instrument was equipped with an argon (488 nm, 250 mw) and a helium-neon (632.8 nm, 0.8 mw) laser. Optical alignment of the instrument was checked daily with the use of fluorescein-labeled latex beads (Polyscience). A constant gain setting of 2.55 produced a mean channel fluorescence (MCF) value of 216 ± 4 (SEM; n = 66) channels on a 1–1000 channel display. Gain for immunofluorescence was also monitored daily with the use of fluorescein-stained calf thymocytes (Fluortrol, Ortho Diagnostic Systems). The Fluortrol was run in neutral PBS containing 1% paraformaldehyde. The gain setting was adjusted to give an MCF value of 155. In 44 experiments the gain settings to achieve this MCF value averaged 4.15 ± 0.01 (SEM). Discrimination of neutrophils from other leukocytes was accomplished in terms of axial light loss and right angle blue light scatter. Fluorescence of stained cells was quantified in terms of right angle green fluorescence greater than 530 nm read on a linear scale. All assays of neutrophils involved examination of 10^4 cells. For all assays of control and patient neutrophils, cells staining negatively for CR3 expression (with fluorescence ⩽ nonspecific fluorescence) were consistently ⩽1% of total cells examined, and no correction was made for this overlap.

Results

Methodologic Considerations

Berger et al[12] have reported that CR3 expression on isolated human neutrophils increases spontaneously upon warming. We have observed the same influence of temperature on expression of CR3 for neutrophils in anticoagulated whole blood from healthy donors, but not for cells from patients with thermal injury. Data summarized in Figure 1 illustrate this difference. For this experiment, blood specimens were obtained from 2 control donors and from 2 patients. Patient specimens were obtained during the first week after injury. All specimens were cooled immediately to 4 C. After enumeration of neutrophils, each specimen was diluted as described and divided into two aliquots of equal volume. One sample of each specimen was incubated at 4 C and the other at 25 C for the next hour before staining at 4 C. The 25 C temperature was chosen to mimic the ambient temperature at which neutrophils are often isolated for study. Comparisons of cold- and warm-incubated control neutrophils for binding of OKM1 antibody, in terms of MCF, showed an increase of 30% and 100% in fluorescence staining. Similar comparisons of patient neutrophils showed no temperature-related increase in fluorescence staining. Because this phenomenon would eliminate or minimize differences in expression of CR3 between control and patient neutrophils, all blood specimens drawn for the studies de-

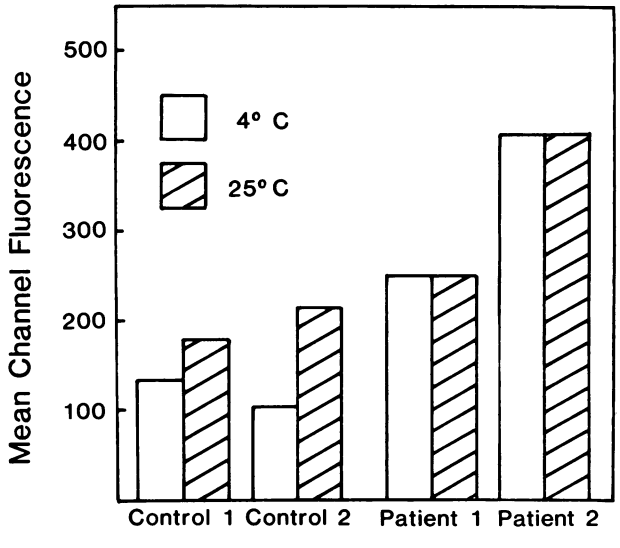

Figure 1—Influence of temperature on binding of OKM1 antibody by neutrophils from control donors and patients with thermal injury. Blood specimens were immediately cooled to 4 C. Neutrophil concentration was adjusted to 5 × 10^6/ml. Each specimen was divided into two aliquots of equal volume, one being incubated at 4 C and other at 25 C for 1 hour before staining at 4 C for expression of CR3. Binding of OKM1 antibody was assessed by flow cytometric analysis in terms of mean channel fluorescence.

Table 2—Reproducibility of Binding of OKM1 Anti-CR3 Antibody by Neutrophils From Three Control Subjects Tested Three Times at Monthly Intervals

	Mean channel fluorescence			
	Date 1	Date 2	Date 3	Mean ± 1 SD
Donor 1	160	162	158	160 ± 2
Donor 2	88	102	124	105 ± 18
Donor 3	120	118	135	124 ± 9

scribed in this report have been processed at 4 C and without preliminary physical separation of the leukocyte subpopulations.

Binding of OKM1 Antibody by Control Neutrophils

For neutrophils from a total of 17 healthy control donors, the MCF values for binding of OKM1 antibody averaged 155 ± 43 (1 SD) (range, 83–216). To determine the constancy of neutrophil CR3 expression for healthy donors over time, binding of OKM1 antibody was assessed three times at approximately 1-month intervals for each of 3 control donors. Data summarized in Table 2 illustrate that expression of CR3 was not grossly variable over this time period in the absence of disease: the standard deviation of the three MCF values obtained for neutrophils from each individual was never >20% of the respective average MCF value.

Binding of OKM1 Antibody by Patient Neutrophils

Like control neutrophils, virtually all patient neutrophils stained positively for CR3 expression. Data in Figure 2A–D illustrate temporal changes observed in binding of OKM1 antibody for neutrophils from 4 of the 8 patients studied. The composite histograms displayed for each patient represent intensities of antibody binding on the days postburn (DPB) noted. This figure is provided to illustrate that patient neutrophils show a unimodal and skewed shift to the right in fluorescence intensity related to increased expression of CR3. Recording of mean channel fluorescence for each assay therefore appropriately reflects the absence of discrete subpopulations of OKM1-positive neutrophils, though does not fully reflect the considerable differences which can occur in the upper limits of OKM1 binding.

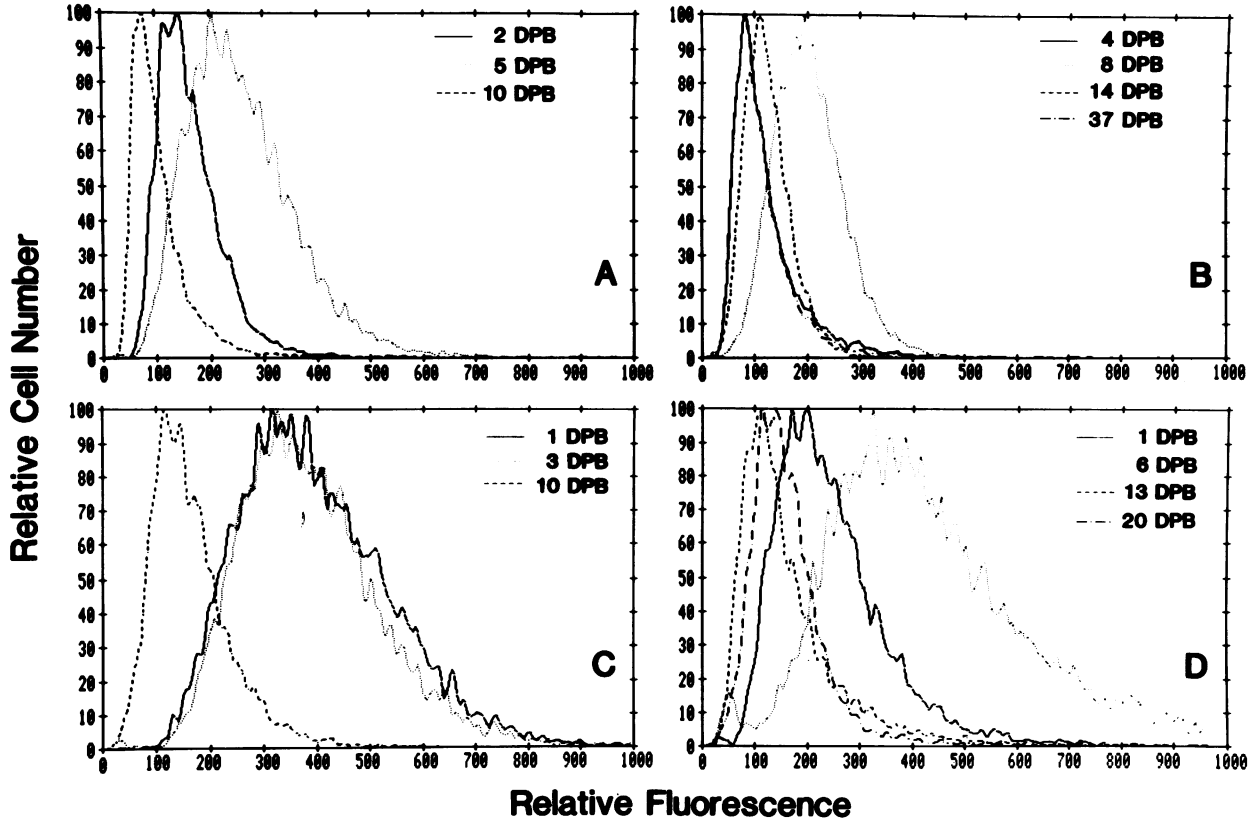

Figure 2—Binding of OKM1 antibody by neutrophils from patients with minor thermal injury without inhalation injury or sepsis. Histograms derived from 4 to 7 such patients studied are presented. Histograms recorded for patients A through D represent antibody binding on the days postburn (DPB) identified. Note that the distributions of fluorescence levels are unimodal at all time points and that they become skewed to the right in association with increased levels of antibody binding. MCF values are presented in the subsequent figure.

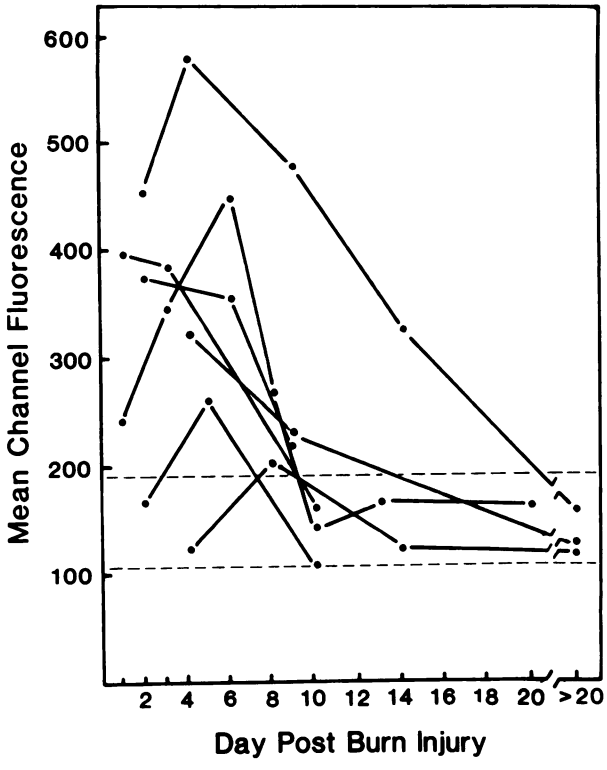

Figure 3—Relationship of binding of OKM1 antibody to DPB injury for neutrophils from 7 patients with minor thermal injury without inhalation injury or sepsis. Binding of antibody is expressed as MCF. The *horizontal dotted lines* define the mean ± 1 SD MCF values for binding of OKM1 antibody by neutrophils isolated from 17 healthy control subjects.

Summarizing the OKM1 binding data for the 7 nonseptic patients in the format represented by Figure 3 better illustrates the temporal nature of changes in CR3 expression. For these patients, the lowest MCF values averaged 146 ± 37 (1 SD) (range, 109–219). This summary value did not differ significantly from that calculated for the 17 control donors ($P > 0.9$). For 2 patients the peak MCF value was observed for neutrophils assayed 1 or 2 DPB. For the other 5 patients, the peak MCF values were observed for neutrophils assayed 4–8 DPB. The peak MCF values for all 7 patients averaged 370 ± 125 ($P < 0.01$ relative to the average lowest values). Comparing the lowest and highest MCF values for each patient reveals an average increase in MCF of 150% (range, 70–260%).

One patient differed from the others in this study in terms of having experienced inhalation injury and sepsis. We present the results of our study of this patient because they differ in one respect from those described above and thereby represent another pattern of change in expression of neutrophil CR3 associated with thermal injury. Figure 4 illustrates that neutrophils assayed 2 DPB exhibited an increase in binding of OKM1 antibody reflected by an MCF value of 452. The MCF values for binding of the antibody by neutrophils assayed on the next five days, 9–31 DPB, remained elevated with a range of 364–427. That these results did not simply reflect a unique normally high level of expression of CR3 by neutrophils from this individual is illustrated by the lower, normal level of antibody bound by neutrophils assayed 60 DPB: MCF = 146. None of the other 7 patients studied exhibited such a prolonged elevated level of expression of neutrophil CR3.

Binding of OKM1 Versus OKM10 Antibody

Wright et al[11] have identified monoclonal antibodies that react with four distinct antigenic determinants of the human leukocyte C3bi receptor. Among these, OKM1 was determined to bind to a site remote from the C3bi-binding site, whereas OKM10 reacts with the ligand-binding aspect of the receptor. Our choice of OKM1 antibody to monitor CR3 expression was based upon this difference, to eliminate the possibility that C3bi bound to the receptor would mask measurement of its up-regulation. Use of only OKM1 antibody for this purpose, however, introduced another difficulty—analysis of binding of OKM1 antibody might not discriminate between increased expression of the epitope recognized by OKM1 antibody and up-regulation of functional receptor.

To establish whether increased binding of OKM1 antibody reflects mobilization of functional CR3, we have compared binding of OKM1 and OKM10 antibodies by unstimulated and stimulated control and by patient

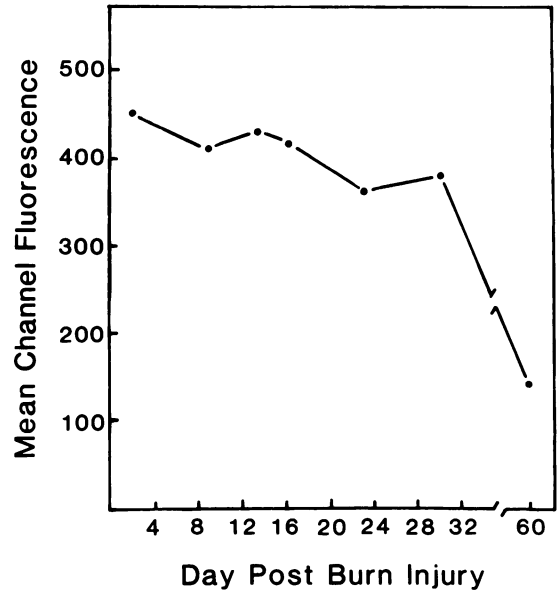

Figure 4—Binding of OKM1 antibody by neutrophils from a patient with minor thermal injury, inhalation injury, and sepsis. MCF values obtained on 7 dates after injury are recorded.

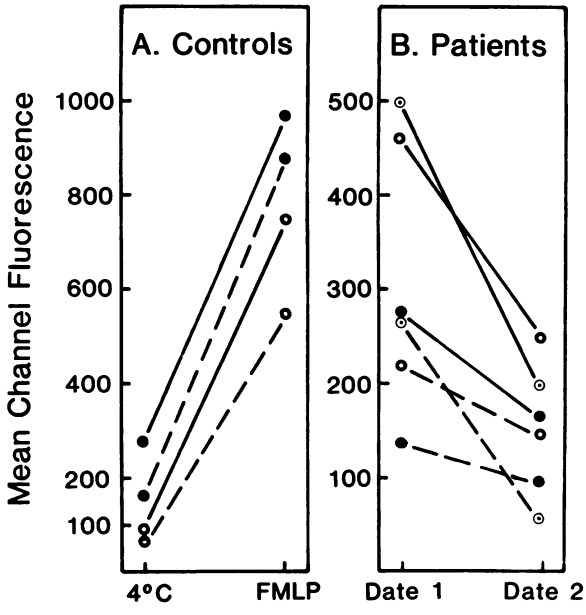

Figure 5—Comparison of binding of OKM1 and OKM10 antibodies by control and patient neutrophils. These antibodies recognize two separate epitopes of human CR3. Antibody OKM1 reacts with a site remote from the ligand-binding site; OKM10 reacts with the ligand-binding aspect of the receptor. Binding of the antibodies to neutrophils was compared in two situations. **A** illustrates the influence of exposure of control neutrophils to F-Met-Leu-Phe (10^{-8} M) on subsequent binding of the two antibodies. *Solid* and *open* data points denote individual cell sources; solid and dashed lines denote binding of OKM1 and OKM10 antibody, respectively. **B** illustrates temporal changes in binding of the two antibodies by neutrophils from patients with thermal injury. Data points are coded to denote results obtained from each of three patients; *solid* and *dashed lines* denote binding of OKM1 and OKM10 antibody, respectively. Both antibodies were applied at saturating concentrations.

neutrophils. Data in Figure 5, panel A illustrate that exposure of neutrophils from 2 control donors to F-Met-Leu-Phe produced an increase in binding of the OKM1 and OKM10 antibodies. Data in panel B illustrate that neutrophils from 3 patients, each studied on two dates, showed a decrease in binding of OKM10 as binding of OKM1 antibody normalized after injury. These concordant trends in binding of the two antibodies by control and patient cells support our application of OKM1 antibody for monitoring expression of neutrophil CR3 in this situation. Comparing control and patient neutrophils in terms of OKM1:OKM10 binding ratio could additionally be interpreted to suggest that not all patient neutrophil C3bi receptors identified by binding of OKM1 antibody are able to react with OKM10 antibody. Implications of this observation will be considered further in the Discussion section of this report.

Discussion

In this report we describe temporal changes in expression of the human neutrophil C3bi receptor, CR3, which occur after minor degrees of thermal injury. Prospective monitoring of expression of CR3 on neutrophils from 7 nonseptic patients revealed a consistent time-related pattern of up-regulation and normalization of expression of the receptor. While the data available do not provide for a precise description of the temporal aspects of this pattern or precise determinations of receptor numbers, they do allow some preliminary conclusions about this response to thermal injury.

Up-regulation of expression of neutrophil CR3 occurred relatively early after injury (Figure 3). For neutrophils from 2 patients first sampled within 24 hours after injury, the mean channel fluorescence (MCF) values, reflecting binding of OKM1 anti-CR3 antibody, were 245 and 395. These values exceeded the average (154) and range (83 to 216) of MCF values observed for neutrophils from 17 healthy controls. For neutrophils from 2 of 3 patients first sampled 2 days after injury, the MCF values were 373 and 455, likewise well outside of the control range. Until additional experiments are completed, we can only speculate about the time after injury when neutrophils first become stimulated to mobilize CR3 and the strength and duration of this stimulus. Given the gross increase in MCF observed at 24 hours after the burn, CR3 may accumulate on the cell surface over time under the influence of lower concentrations of stimulus, beginning very early after injury. Alternatively, given the short time required for mobilization of CR3 on control neutrophils exposed to a stimulus *in vitro* (<30 minutes), one could argue that a doubled or tripled MCF value reflects a response of neutrophils closer timewise to the appearance of a more concentrated stimulus after injury. Resolution of these alternatives will be complicated, however, because CR3 mobilized to the cell surface is measurable well after the time of stimulation because of the apparent stability of the new phenotype.[12]

The time of peak expression of neutrophil CR3 was variable among the patients studied. For 3 patients the peak MCF value was observed 1 or 2 days after injury. For the others the peak MCF values occurred during Days 4–8 after injury. These results suggest that peak mobilization of neutrophil CR3 occurs essentially within the first week after minor thermal injury and that the upward limit of this response can approximate a quadrupling of receptor number.

Normalization of MCF values began to occur for 6 of the 8 patients within 7 days after injury. The time to reach normality (MCF ≤216) varied from 3 to more than 20 days. The basis for such variability among the nonseptic patients was not obvious. The prolonged elevation of expression of CR3 on neutrophils from a patient with inhalation injury and sepsis, however, preliminarily identifies one factor influencing this

phenomenon (Figure 4). For the latter patient, the MCF value was elevated on the second day after injury and remained elevated on the next five assay dates, including 31 days after injury. Although damage to pulmonary tissue alone might have produced this unusual neutrophil response, it is more likely that sepsis was the primary cause. Our guess is based upon our recent failure to observe the same extended elevation of neutrophil CR3 in a patient with inhalation injury without septic complications.

The level of mobilization of neutrophil CR3 following minor thermal injury appears to approximate that which can be achieved by exposure of control neutrophils to a stimulus *in vitro*. For 5 of the 8 patients studied, maximum MCF values >300 were observed. Such values approximate those obtained for control neutrophils exposed to f-Met-Leu-Phe *in vitro*. The MCF values for binding of OKM1 antibody by cells from 6 healthy donors incubated at 37 C for 20 minutes in the absence of stimulus averaged 141 ± 22 (I SD) (range, 115 to 174); the MCF values for cells incubated under the same conditions in the presence of 10^{-5} M tripeptide averaged 437 ± 123 (range, 291–608). This comparison would suggest that the strength of the *in vivo* stimulus for mobilization of neutrophil CR3 following minor thermal injury is comparable to that provided by 10^{-5} f-Met-Leu-Phe. Given the association of CR3 with adherence functions of the neutrophil,[15] it must also be recognized that this analysis may not reflect CR3 expression on all neutrophils in the peripheral circulation—cells with greater levels of CR3 expression may have been marginated in these patients and thereby excluded from analysis.

The mechanism of increased expression of neutrophil CR3 in other situations has been determined by several investigators to reflect mobilization of receptors from an intracellular pool[12–14] associated with specific cytoplasmic granules.[13,14] Receptors are transferred to the cell surface as these granules fuse with the cell membrane in response to a stimulus of secretion. That the increase in expression of neutrophil CR3 following thermal injury involved recruitment from an intracellular pool is supported in part by our observation that neutrophils from such patients do not exhibit temporal changes in the expression of a nonpolymorphic cell surface determinant identified by W6/32 antibody[16] (McCormack et al, manuscript in preparation). Also supporting an association of patient neutrophil CR3 mobilization with stimulated secretion is the evidence reported by Alexander[17] that neutrophil secretory function is stimulated *in vivo* after thermal injury.

Still missing from a full description of this injury-related phenomenon is identification of the stimulus or stimuli involved. Recent evidence of complement activation following thermal injury[18] suggests that C5a and C5a des Arg may be major stimuli for up-regulation of neutrophil CR3 in this situation. Also supporting this relationship is the finding that CR3 is up-regulated for control neutrophils exposed *in vitro* to C5a des Arg and for neutrophils from patients undergoing hemodialysis under conditions demonstrated to provide for complement activation.[15] The possibility must be reserved, however, that multiple secretagogues may be produced at the same or different times after the thermal insult.

We have presented data (Figure 5) to support our application of OKM1 antibody to monitor expression of neutrophil CR3 after thermal injury. Although our analysis of these data emphasized only trends in binding of OKM1 and OKM10 antibodies by control and patient neutrophils, less casual scrutiny of the data provides other potentially useful information. One can observe that control neutrophils appear to bind comparable amounts of OKM1 and OKM10 antibody and that patient neutrophils appear to bind less OKM10 than OKM1 antibody. These differences would suggest that some of the receptors on patient neutrophils might be occupied by C3bi to inhibit binding of OKM10 antibody. Although the presence of ligand will have to be confirmed, the possibility of contemporary mobilization of CR3 and down-regulation of functional receptor demonstrates the need for and potential utility of application of both antibodies in future studies of expression and function of neutrophil CR3 *in vivo*.

What is the functional significance of mobilization of neutrophil CR3 in response to thermal injury? Given the role of CR3 in binding of C3bi as an opsonin for phagocytosis, up-regulation of CR3 on circulating neutrophils would be expected to prepare or prime the cells for phagocytic activity on arrival at a site of tissue trauma or infection. Up-regulation of CR3 might be of no benefit, however, should excess soluble C3bi be available to functionally inactivate a proportion of these receptors. Evidence that the C3bi receptor, or at least its Mol epitope, has a further role in adherence properties of the neutrophil[13] would suggest that mobilization of CR3 would also stimulate adherence of the circulating cell to vascular endothelium, to promote exit of cells from the circulation. It is also possible, however, that some level of enhancement of expression of the receptor *in vivo* might mediate excessive margination of circulating neutrophils to the disadvantage of the patient.

We suggest that further studies of modulation of neutrophil CR3 expression are warranted as a measure of microbicidal and adherence properties of patient neutrophils and especially as a sensitive indirect measure of generation of stimuli of neutrophil secretory func-

tion *in vivo*. Our own efforts are now being extended to identification of the secretagogue(s) generated in response to thermal injury and to characterization of patterns of mobilization of neutrophil CR3 associated with more severe degrees of thermal injury. Preliminary information available suggests that still other patterns of modulation of this leukocyte receptor will ultimately be discovered.

Note Added in Proof

During the period this manuscript was in review, a similar report appeared in the New England Journal of Medicine (1986, 314:948–953). In that report Moore et al provide evidence for augmented expression of both CR3 and CR1 (C3b receptor) on neutrophils isolated from thermal injury patients.

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