Platelet Activation by Immobilized Monoclonal Antibody: Evidence for a CD9 Proximal Signal

By Lee Griffith, Joseph Slupsky, Jutta Seehafer, Lynn Boshkov, and Andrew R.E. Shaw

Anti-CD9 monoclonal antibodies (MoAbs) are reported to attach to polystyrene latex beads. Cross-linking F(ab')2 fragments of the anti-CD9 MoAb 50H.19 induce dense-granule release and dose-dependent platelet aggregation when attached to poly styrene latex beads. Cross-linking F(ab')2 fragments of MoAb 50H.19 by F(ab')2 fragments of goat antimouse IgG does not result in platelet aggregation unless the second antibody is bound to latex beads, indicating that immobilization, and not cross-linking of the stimulus, is critical to the initiation of the CD9 signal. In contrast, F(ab')2 fragments of the second antibody readily induce the aggregation of platelets treated with the anti-FcγII receptor MoAb IV.3. Immobilization of MoAb per se is insufficient to induce an activation signal because intact and F(ab')2 fragments of nonstimulatory MoAb directed to glycoprotein Ib and HLA class I do not become stimulatory when attached to beads. CD9-induced activation requires cytoskeletal rearrangement because it is inhibited by cytochalasin B. Aggregation is blocked by inhibitors of the thromboxane pathway, indicating that CD9 activates phospholipase C indirectly through prior activation of phospholipase A2.

Although the precise function of the human cell surface glycoprotein CD9 is unknown, the striking ability of monoclonal antibodies (MoAbs) of the CD9 cluster to promote granule secretion and rapid platelet aggregation suggests direct participation of CD9 in the initiation of signals leading to platelet activation. Of 107 antiplatelet MoAbs examined at the platelet workshop of the IV Leucocyte Typing Conference in Vienna, only 10 induced rapid platelet aggregation, of which nine were directed against CD9, suggesting that CD9 may be unique among platelet surface proteins in the potency with which it supports antibody-induced platelet activation. CD9 may therefore be a signal-initiating molecule. MoAbs to CD9 have been shown to stimulate intrinsic protein phosphorylation, ADP ribosylation, phosphoinositide hydrolysis, diacylglycerol formation, intracellular Ca2+ flux, and thromboxane synthesis, supporting the possibility that CD9 is involved in the induction of metabolic events under the regulation of phospholipase C. In most of these studies it was implicitly assumed that the antibody exerted its effect through stimulation of CD9. Recently, this assumption was challenged by the inability of soluble F(ab')2 fragments of anti-CD9 MoAb to induce platelet activation and by the ability of MoAb directed to the FcγRII receptor to prevent platelet aggregation and cytosolic calcium flux, suggesting that the signalling function may be mediated entirely through stimulation of the Fc receptor. We present here evidence that CD9 can initiate a platelet activation signal, but has a unique requirement that is not fulfilled by cross-linking. We show that nonstimulatory F(ab')2 fragments of the anti-CD9 MoAb 50H.19 become potent platelet agonists when attached to poly styrene latex beads, and that the CD9 proximal signalling pathway is pharmacologically distinguishable from that induced by the intact MoAb.

Materials and Methods

Platelet preparation. Venous blood was collected from volunteers who denied taking medication for the 2 previous weeks. The blood was drawn into citrated vacutainer tubes and platelet-rich plasma (PRP) collected by centrifugation at 150g for 10 minutes at ambient temperature. Apyrase (0.1 U) (grade V; Sigma, St Louis, MO) and 10 ng/mL of prostacyclin (Sigma) were added per milliliter. Platelets were concentrated by centrifugation at 700g for 10 minutes and resuspended in 0.5 mL of Tyrode's-HEPES buffer containing 0.06 U/mL apyrase and 0.1% bovine serum albumin (BSA). Platelets were separated by passage over a 15-mL Sepharose 2B column (Pharmacia, Montreal, Quebec, Canada). The collected platelets were diluted to 5 mL and their concentration adjusted to 2 × 10^10 platelets/mL.

Platelet aggregation. Platelet aggregation was performed using column-washed platelets at 37°C in a Payton aggregometer (Payton, Scarborough, Ontario, Canada). All aggregation assays except those using thrombin were performed in the presence of 0.36 mg/mL of fibrinogen. The ADP scavenging system phosphocreatine/creatinine phosphokinase (CP/CPK) was used at 5 mmol/L and 40 U/mL of CP and CPK, respectively. The thromboxane inhibitor BM13.177 (the kind gift of Dr Stegmeier, Boehringer-Mannheim, Germany) was used at 10 μmol/L and the cyclooxygenase inhibitor indomethacin at 20 μmol/L. Changes in light transmission induced in 2 × 10^9 stirred platelets in a volume of 0.5 mL were used to quantitate aggregation. The reduction in light transmission due to the opacity of the latex beads was corrected by restoration of the baseline.

5HT secretion. PRP containing 0.1 U/mL of apyrase but no prostacyclin were loaded with 5 μCi/mL [3H]5HT (serotonin) of specific activity 26 Ci/mmol (NEN Biochemicals, Mississauga, Ontario, Canada) for 1 hour at 37°C. Release of [3H]5HT into the supernatant after exposure to MoAb for 4 minutes was quantitated by fixation of the platelets with 6% glutaraldehyde 10 mmol/L EGTA in phosphate-buffered saline (PBS) to terminate release, pelleting at 10,000g, and liquid scintillation counting of the supernatant. The results are expressed as a percentage of the total [3H]5HT determined by lysis with the detergent NP-40 (0.5%). Background release by nonstimulated platelets was determined over the same time course.

From the Department of Medicine, University of Alberta, Edmonton, Alberta; the Cross Cancer Institute, Edmonton, Alberta; and the Canadian Red Cross Blood Transfusion Service, Edmonton, Alberta.


Supported by grants from the Research Initiative Program of the Alberta Cancer Board, and the Medical Research Council of Canada. L.G. is the recipient of the McCartney Postdoctoral Fellowship.

Address reprint requests to Andrew Shaw, PhD, Department of Medicine, Cross Cancer Institute, 11560 University Ave, Edmonton, Alberta, Canada T6G 1Z2.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1991 by The American Society of Hematology.

0006-4971/91/7807-005$3.00/0

Blood, Vol 78, No 7 (October 1), 1991: pp 1753-1759

1753
MoAb and antisera. MoAb 50H.19 is an IgG2a directed against CD9. MoAb 9H.1 is an IgG1 reactive with β2 microglobulin. MoAb AP-1 is directed against glycoprotein Ib (GPIb) and was kindly provided by Dr Robert Montgomery (The Blood Center, S.E. Wisconsin). MoAb SZ-1 directed against GPIb-GPIX is an IgG2a (Bio/can Scientific, Mississauga, Ontario, Canada). All MoAbs were purified on Protein-A Sepharose (Pharmacia) before use. A horseradish peroxidase Fc-specific goat antimeouse IgG Fc-specific antiserum was purchased from Originon Teknika (West Chester, PA) and F(ab')2 fragments of a goat antimeouse IgG (heavy and light chain) antiserum (Serotec) were purchased from Prince Laboratories (Toronto, Ontario, Canada).

Preparation of antibody-coated latex beads. One microliter of 0.8-μm diameter polystyrene latex beads (LB-8, Sigma) were washed three times with Tyrodes-HEPES buffer and incubated with a 200 μL solution of either F(ab')2 fragments of MoAh (0.7 mg/mL) or with F(ab')2 fragments (0.7 mg/mL) of goat antimeouse IgG for 1 hour at 23°C with rotation. The beads were pelleted, resuspended in 3% BSA for 1 hour at 23°C on a rotary shaker, and washed three times in Tyrode's-HEPES buffer. Control beads were treated with BSA and washed three times. The beads were resuspended in a final volume of 50 μL of Tyrode's-HEPES buffer. Nominal concentrations of antibody fixation by the beads was determined by spectrophotometric determination of antibody concentration remaining after this procedure.

RESULTS

Purity of F(ab')2 fragments. To test whether the F(ab')2 fragments of MoAb 50H.19 were contaminated with Fc fragments that might interact with the FcγII receptor, intact IgG and Fc-depleted F(ab')2 fragments of MoAb 50H.19 were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted using horseradish peroxidase-conjugated Fc region-specific IgG (Organon Teknika). The F(ab')2 fragments were greater than 97% pure by the criteria of protein-staining (Fig 1, lane 4) and reactivity with an Fc-specific antibody (Fig 1, lane 2) in comparison with the uncleaved MoAb (Fig 1, lane 1).

MoAb 50H.19-induced activation is inhibitable by MoAb IV.3. The aggregation of gel-filtered human platelets induced by intact MoAb 50H.19 (Fig 2) was completely inhibited by preincubating the platelets with 10 μg/mL of the anti-FcγII receptor MoAb IV.3 (Fig 2), indicating that occupation of the Fc receptor is an obligatory requirement for generation of an anti-CD9 MoAb-induced activation signal as previously reported for the anti-CD9 MoAb SYB-1. MoAb IV.3 alone did not stimulate the platelets (Fig 2), nor did it block the response to thrombin, suggesting that its inhibitory activity was mediated through blockade of the Fc receptor (Fig 2).

F(ab')2 fragments of MoAb 50H.19 are nonstimulatory and nonimmobilized. F(ab')2 fragments of MoAb 50H.19 did not stimulate platelet aggregation (Fig 3), as previously described for MoAb SYB-1. However, presentation of the fragments on the surface of polystyrene latex beads resulted in rapid and complete platelet aggregation (Fig 3).
activation could be competitively inhibited by soluble F(ab')2 fragments of MoAb 50H.19, indicating that it was mediated through stimulation of CD9 (Fig 3). Latex beads exposed to BSA did not activate platelets, indicating that the beads themselves were incapable of inducing an activation signal (results not shown). Preincubation of the platelets with MoAb IV.3 had no effect on their activation by immobilized F(ab')2 fragments of MoAb 50H.19 (Fig 3), precluding the possibility that the signal was elicited by contamination with uncleaved IgG. The number of beads added to the platelets and the degree of aggregation they induced showed a dose-response relationship (Fig 4). Although we did not attempt to quantitate the number of active MoAb binding sites per bead, we estimated the amount of MoAb bound per bead by spectrophotometrically measuring the protein concentration of the antibody solution before and after adsorption to the beads. By this criterion, which would overestimate rather than underestimate the effective concentration of bound MoAb, the dose-response relationship was similar to that established for soluble intact anti-CD9 MoAb.6,15

Immobilization of F(ab')2 fragments of MoAb 50H.19 induces platelet granule release. The ability of intact, and F(ab'), fragments of MoAb 50H.19 to induce 5HT release from dense granules was evaluated (Table 1). Soluble F(ab')2 fragments of MoAb 50H.19 did not release 5HT over background (Table 1), as reported for F(ab')2 fragments of other agonistic anti-CD9 MoAbs.13,14 However, when the F(ab')2 fragments of MoAb 50H.19 were immobilized on polystyrene latex beads, 23% of total 5HT was released over background. In contrast, activation by intact MoAb 50H.19 resulted in 60% release. Because the height of the aggregation curves were similar, it suggests that

<table>
<thead>
<tr>
<th>Activator</th>
<th>Inhibitor</th>
<th>% 5HT released (± 1 SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MoAb 50H.19</td>
<td>CP/CPK</td>
<td>62.5 ± 2.6</td>
</tr>
<tr>
<td>MoAb 9H.1</td>
<td></td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>F(ab')2 50H.19</td>
<td></td>
<td>3.9 ± 0.9</td>
</tr>
<tr>
<td>F(ab')2 50H.19 (I)</td>
<td></td>
<td>26.6 ± 6.9</td>
</tr>
<tr>
<td>F(ab')2 50H.19 (I)</td>
<td>CP/CPK*</td>
<td>1.4 ± 1.0</td>
</tr>
<tr>
<td>F(ab')2 50H.19 (I)</td>
<td>BM13.177t</td>
<td>3.9 ± 2.8</td>
</tr>
<tr>
<td>F(ab')2 50H.19 (I)</td>
<td>Indomethacin#</td>
<td>2.2 ± 1.3</td>
</tr>
<tr>
<td>Polystyrene beads</td>
<td></td>
<td>4.3 ± 1.5</td>
</tr>
<tr>
<td>Unstimulated platelets</td>
<td></td>
<td>3.7 ± 0.6</td>
</tr>
</tbody>
</table>

5HT release was determined by measuring the levels of [3H]-5HT in the supernatants of platelets activated by exposure to MoAb and expressed as percentage of total 5HT. Inhibitors were preincubated 5 minutes before testing.

Abbreviation: (I), immobilized antibody.
*CP/CPK 5 mmol/L/40 U/mL.
†BM13.177 10 μmol/L.
‡Indomethacin 20 μmol/L.
immobilized F(ab')2 fragments provide a weaker stimulus for dense granule release, perhaps through selective activation of signals effecting degranulation. 5HT was not released by the nonstimulatory control MoAb 9H.1 (Table 1). The presence of either the ADP scavenger creatine phosphate/creatine phosphokinase (CP/CPK), the selective nonprostanoic thromboxane antagonist BM13.177,17 or the cyclooxygenase inhibitor indomethacin completely blocked 5HT secretion, suggesting that the release reaction, in common with aggregation, requires both secretion of ADP and stimulation of the thromboxane pathway (Table 1).

**Immobilization and not cross-linking of CD9 is required for activation.** Cross-linking nonstimulatory F(ab')2 fragments of an anti-CD9 MoAb with an intact goat antimouse IgG as the second antibody is reported to cause platelet activation,13 suggesting that occupation of the FcyII receptor by the Fc region of the second antibody is the trigger. However, it cannot be ruled out that cross-linking of CD9 by the second antibody may also contribute to the response. To determine whether cross-linking CD9 alone is able to initiate a platelet-activating signal, F(ab')2 fragments of MoAb 50H.19 were cross-linked by addition of F(ab')2 fragments of a goat antimouse IgG antiserum. No aggregation was observed (Fig 5), indicating that cross-linking of CD9 is insufficient to elicit a signal. Addition of F(ab')2 fragments of the goat antimouse IgG antibody to platelets exposed to MoAb IV.3, however, promoted strong and rapid platelet aggregation (Fig 5), showing that the F(ab')2 fragments of the goat antimouse IgG could effectively cross-link murine MoAb, and suggesting that the FcyII receptor is qualitatively different from CD9 in its requirement for triggering. We reasoned that if immobilization of CD9 was required to initiate a signal, then attachment of the F(ab')2 fragments of the second antibody to polystyrene latex beads would activate platelets in the presence of F(ab')2 fragments of MoAb 50H.19. Attachment of the F(ab')2 fragments of the goat antimouse IgG to latex beads did indeed result in a rapid and strong aggregation response (Fig 5), supporting the postulate that immobilization is a critical requirement for initiation of the CD9 signal. To test whether platelets inherently respond to the immobilization of any potential antibody-binding site, MoAb against GPIb (AP-1), GPIb-GPIX (SZ-1), and HLA class I (9H.1) were exposed to MoAb IV.3, however, promoted strong and rapid platelet aggregation (Fig 5), showing that the F(ab')2 fragments of the goat antimouse IgG could effectively cross-link murine MoAb, and suggesting that the FcyII receptor is qualitatively different from CD9 in its requirement for triggering. We reasoned that if immobilization of CD9 was required to initiate a signal, then attachment of the F(ab')2 fragments of the second antibody to polystyrene latex beads would activate platelets in the presence of F(ab')2 fragments of MoAb 50H.19. Attachment of the F(ab')2 fragments of the goat antimouse IgG to latex beads did indeed result in a rapid and strong aggregation response (Fig 5), supporting the postulate that immobilization is a critical requirement for initiation of the CD9 signal. To test whether platelets inherently respond to the immobilization of any potential antibody-binding site, MoAb against GPIb (AP-1), GPIb-GPIX (SZ-1), and HLA class I (9H.1) were attached to polystyrene beads either as intact MoAbs, or, in the case of SZ-1, as F(ab')2 fragments (Table 2). None of the MoAbs or their fragments became stimulatory as a consequence of attachment to beads, indicating that immobilization of surface molecules per se does not cause signalling, and suggesting that the requirement for immobilization of the stimulus may be unique to CD9. Preincubation of platelets with cytochalasin B, an inhibitor of F-actin polymerization, completely prevented activation by immobilized F(ab')2 fragments (Fig 3), indicating that cytoskeletal assembly is a necessary requirement for CD9-induced aggregation.

**Signalling by immobilized F(ab')2 fragments of MoAb 50H.19 but not by intact antibody has an absolute requirement for both ADP secretion and occupation of the thromboxane receptor.** Stimulation of platelet aggregation by MoAb 50H.19 (Fig 6A) was only partially inhibitable by the ADP scavenger system CP/CPK (Fig 6A) or by the thromboxane receptor blocker BM13.177 (Fig 6B), but was completely inhibited by their combination (Fig 6A) as previously reported for another anti-CD9 MoAb.18 This finding suggests that the anti-CD9 MoAb is generating parallel activation signals.19 The relative insensitivity of aggregation induced by the intact MoAb to inhibitors of the thromboxane pathway is similar to that reported for the aggregation induced by cross-linking of the Fc receptor18 and is consonant with the requirement for an Fc in the stimulatory

---

**Table 2. Effect of Immobilization of the MoAb on Induction of Platelet Aggregation**

<table>
<thead>
<tr>
<th>MoAb Specificity</th>
<th>Intact IgG (10 µg/mL)</th>
<th>Intact IgG (l)</th>
<th>F(ab')2</th>
<th>F(ab')2(l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50H.19 Anti-CD9</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9H.1 Anti-HLA class I</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>AP-1 Anti-GPlb</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>SZ-1 Anti-GPlb-GPIX</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Platelet aggregation was determined on 2 x 10^6 platelets in 0.5 mL after the addition of either the intact MoAb or MoAb-coated polystyrene latex beads.

Abbreviations: (l), 10 µg/mL equivalent of MoAb immobilized on polystyrene latex beads; +/-, aggregation; NT, not tested.
MoAb. In contrast, when platelets were activated by immobilized F(ab')2 fragments of MoAb 50H.19, the aggregation was completely prevented by either the thromboxane receptor antagonist BM13.177 (Fig 6B) or by the ADP scavengers CP/CPK (Fig 6B), implicating an absolute requirement for the activation of both pathways. The CD9 signal therefore appears to be qualitatively different from that induced by the intact MoAb and to specifically activate phospholipase A2 independently and before the activation of phospholipase C.

**DISCUSSION**

We show here that platelets can be activated by immobilized F(ab')2 fragments of anti-CD9 MoAb by a mechanism that is not inhibitible by Fc receptor blockade. This finding shows for the first time that CD9 can initiate a platelet-activating signal in the absence of Fc receptor stimulation, and implies that immobilization of CD9 by the activating stimulus may be a key requirement for initiation of an intracellular signal. The immobilized F(ab')2 MoAb-induced response appears to be specific for stimulation of CD9 because it is abrogated by soluble F(ab')2 fragments of MoAb 50H.19. The CD9 proximal signal is characterized by its complete dependence on ADP secretion and a functional thromboxane pathway. Because the thromboxane receptor antagonist BM13.177 and the cyclo-oxygenase inhibitor aspirin only slightly reduce platelet aggregation induced by intact MoAb 50H.19, it would appear that CD9-dependent signalling makes only a minor contribution to the MoAb 50H.19 activating signal, consistent with the observed aspirin-insensitivity of activation by the anti-CD9 MoAb ALB. One hundred and seventy MoAbs were tested in a workshop of the IVth Leukocyte Typing Conference for their ability to promote platelet aggregation. Eighteen MoAbs induced some degree of platelet aggregation, of which six were directed against GPIIb/IIIa and 10 against CD9, suggesting that GPIIb/IIIa and CD9 may share a common property that allows for efficient stimulation of the Fc receptor. We have reported on the basis of chemical cross-linking studies that CD9 and GPIIb/IIIa physically associate on the platelet surface under conditions of anti-CD9 MoAb stimulation, a possibility further supported by the ability of anti-GPIIb/IIIa MoAbs to block platelet activation by anti-CD9 MoAb. GPIIIb/IIIa is known to cluster on occupation of the ligand-binding region, possibly through exposure of new sites for protein-protein interaction. It is probable therefore that CD9 and GPIIb/IIIa cocluster under appropriate stimulation, thereby achieving the local IgG concentration required to trigger the Fc receptor.

The need to immobilize either the primary or secondary antibody on a rigid support to achieve effective stimulation...
through CD9 suggests to us the possibility that CD9 might be a component of a receptor system physiologically responsive to a mechanically rigid stimulus such as extracellular matrix. Our inability to produce even a small amount of platelet aggregation by cross-linking soluble F(ab’), fragments of an anti-CD9 MoAb with F(ab’), fragments of a goat antimuscle IgG, although the latter effectively trigger platelet activation through cross-linking of the Fc receptor, suggests that CD9 and the Fc receptor are qualitatively different in their requirements for stimulation. MoAb directed to other platelet surface determinants immobilized on latex beads either as intact antibody or as F(ab’), fragments did not activate the platelet, indicating that immobilization of membrane proteins per se is not a trigger for generation of a platelet activating signal and emphasizing the specificity of this requirement for CD9. It is of interest that a similar requirement for immobilization rather than cross-linking has been shown for the activation of proliferation in T lymphocytes by MoAb directed against the T-cell receptor molecule CD3, where the requirement may serve to physiologically restrict T-cell proliferation to T-cell–adhesive cell interactions. CD9-induced activation of the platelet, however, leads to the activation of metabolic pathways resulting in expression of an adherent rather than a proliferative phenotype. It is tempting to speculate that the physiologic consequence of the requirement for immobilization of CD9 to initiate a signal might be to restrict induction of adhesion to contact with the extracellular matrix. The notion that CD9 may function in the mechanism of an immobilized stimulus is appealing in the context of evidence for its association with the adherence receptor GPIIb/IIIa and suggests a mechanism by which recognition of a component of the extracellular matrix by an integrin could be coupled through a responsive cytoskeleton to the initiation of intracellular signals effecting enhanced adhesiveness. It is of interest that platelet activation by fibrillar collagen, a major component of the subendothelial basement membrane, also proceeds via an aspirin- and cytochalasin B-sensitive involving the apparent independent activation of phospholipase A2 and phospholipase C. Although the inability of F(ab’), fragments of anti-CD9 MoAb to inhibit the response of platelets to collagen would appear to rule out a role for CD9 in that response, such a role cannot be completely discounted because it has yet to be convincingly demonstrated that F(ab’), fragments of anti-CD9 MoAb are able to inhibit any aspect of platelet activation. The possibility that CD9 performs a signal-initiating function is compatible with the recent molecular cloning data indicating that CD9 is a multiply inserted membrane protein of novel structure containing four putative transmembrane domains. The extensive extracellular domain could function as a ligand-binding site. Although a ligand for CD9 has yet to be identified, our finding that CD9 requires immobilization to signal would predict that such a ligand would be located within the extracellular matrix. Alternatively, CD9 may be triggered indirectly through immobilization of the CD9-associated integrin through attachment to its ligand. Although the details of CD9-dependent signalling have yet to be established, our demonstration of an absolute requirement for a functional thromboxane pathway indicates that CD9 stimulates phospholipase C by the classical agonist route through prior activation of phospholipase A2, rather than through the thromboxane-independent component of Fc receptor signalling induced by the intact MoAb.

In conclusion, the results presented here implicate CD9 as an independent initiator of platelet-activating signals. Further studies will be directed towards an understanding of the involvement of CD9 with members of the integrin family, the identification of possible ligands, and an understanding of the mechanisms of activation of phospholipases regulating metabolic pathways required for expression of the adherent phenotype.

ACKNOWLEDGMENT

We thank the staff of the Audiovisual Department of the Cross Cancer Institute for their help with the preparation of the figures, Dr M. Longenecker (Department of Immunology, University of Alberta, Edmonton, Alberta, Canada) for the use of MoAbs 50H.19 and 9H.1, and Dr C. Anderson (Davis Medical Research Center, Columbus, OH) for MoAb IV.3.

REFERENCES


Platelet activation by immobilized monoclonal antibody: evidence for a CD9 proximal signal

L Griffith, J Slupsky, J Seehafer, L Boshkov and AR Shaw