# AS4.2 CD54 (ICAM-1) cluster report

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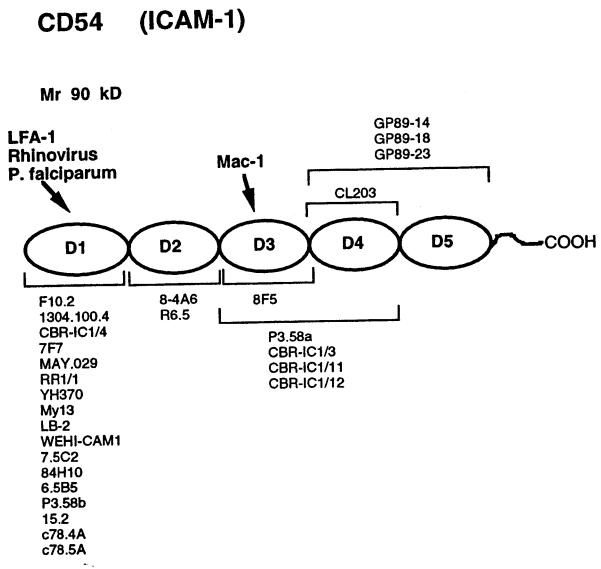


ICAM-1 was initially identified with monoclonal antibody (mAb) RR1/1 as a widely distributed, cytokine-inducible counterreceptor for LFA-1 (CD11a/CD18) [1–3]. ICAM-1 was clustered as CD54 in the Fourth Workshop with mAb RR1/1, 7F7, LB-2, 8F5, WEHI-CAM1, OKT27, F2B1.8, and My13. The first two mAb, RR1/1 and 7F7, were resubmitted as S107 and S098 to the Adhesion Structure Section of the Fifth Workshop along with nine new mAb. CD54 mAb in other panels of the Fifth Workshop included T101 (WEHI-CAM1) in the T-cell section and ICAM-1.1 (D8/H10) and ICAM-1.2 (BL1-4D6) in the B-cell Section. In the Fifth Workshop, transfection studies addressed epitope mapping and its correlation with binding of ICAM-1 to LFA-1 or *Plasmodium falciparum*-infected erythrocytes.

## Cellular expression

Histochemical analysis of lymphoid and other tissues [Autschbach *et al.*, AS2.5; Freedman *et al.*, B30.20; Koretz *et al.*, AS4.16; unpublished Workshop studies by Athannasaou, Bene, Cerf-Bensussan, Malizia, Patarroyo, Soligo, and Timens] confirmed previous work demonstrating highest levels of CD54 antigen on activated endothelium, with lower levels that increase in inflammation on multiple other cell types. One assay that identified all CD54 mAb among the Subpanel 4 mAb was flow cytometric analysis of freshly isolated peripheral blood monocytes compared with monocytes after 1 day in culture [4; Most, unpublished Workshop report]. CD54 and CD106 (VCAM-1) were the sole CAMs present on fetal bone marrow stromal cells and, interestingly, CD106 was upregulated by interleukins IL-1 or IL-4, whereas CD54 expression was increased only by IL-1 [Dittel and Le Bien, AS7/8.15].

## Immunochemistry

The characterization of CD54 as a single-chain glycoprotein of $M_r$ 90 kDa is well established in the literature [3], and this was confirmed for selected mAb from the Fifth Workshop. Other than N-linked carbohydrate, no other posttranslational modifications have been reported for CD54.

## Molecular cloning

The human CD54 cDNA predicts a type 1 integral membrane glycoprotein with an extracellular domain comprised of five immunoglobulin superfamily (IgSF) repeats, a 24-residue transmembrane region, and a 28-amino-acid cytoplasmic domain [6]. Sequences have subsequently become available for mouse, rat, chimpanzee, and dog (Genbank, release 79). CD54 is most closely related structurally and functionally to CD50 (ICAM-3) and CD102 (ICAM-2) with 34 per cent sequence identity in the extracellular region to ICAM-2 and 52 per cent identity with ICAM-3 [5,6]. The gene for CD54 has been characterized [7].

## Transfectant and epitope analysis

Extensive mutational analysis has been published for CD54, localizing the binding sites for LFA-1,

**Table 1** Summary of the inhibitory effects of CD54 mAb

| mAb | | Epitope in domain | Inhibition of binding of† | | | | References‡ |
|---|---|---|---|---|---|---|---|
| Workshop code* | Clone name | | LFA-1 | Rhinovirus | *P. falciparum* | Mac-1 | |
| S107; (IV) M144, B112 | RR1/1 | D1 | Yes | Yes | No | No | 1, 8, 10, 11 |
| S098; (IV) N72, A16 | 7F7 | D1 | No | | Yes | | 10 |
| (IV) M165, B50 | LB-2 | D1 | Yes | Yes | Yes | No | 8–11 |
| (IV) N108 | WEHI-CAM1 | D1 | Yes | | No | | 10 |
| (IV) M57 | My13 | D1 | No | | Yes | | 10 |
| S082 | F10.2 | D1 | Yes | | | | |
| S083 | 1304.100.4 | D1 | | | | | |
| S095 | CBR-IC1/4 | D1 | Yes | | | No | 13 |
| S105 | MAY.029 | D1 | Yes | | | | |
| S116 | YH370 | D1 | Yes | | | | |
| | 7.5C2 | D1 | Yes | | No | | 10 |
| | 84H10 | D1 | Yes | | Yes | No | 10 |
| | 6.5B5 | D1 | Yes | | Yes | | 10 |
| | P3.58b | D1 | No | | Yes | | 10 |
| | 15.2 | D1 | Yes | | Yes | | 10 |
| | c78.4A | D1 | Yes | Yes | | | 14 |
| | c78.5A | D1 | Yes | Yes | | | 14 |
| | R6.5 | D2 | Yes | Yes | No | Yes | 10–13 |
| S100 | 8-4A6 | D2 | Yes | | No | | 10 |
| (IV) M60 | 8F5 | D3 | No | | No | | 10 |
| | P3.58a | D3–D4 | No | | No | | 10 |
| S094 | CBR-IC1/3 | D3–D4 | | | | | § |
| S096 | CBR-IC1/11 | D3–D4 | No | | | Yes | § |
| S097 | CBR-IC1/12 | D3–D4 | | | | | § |
| | CL203 | D4 | No | No | No | No | 8, 11 |
| | GP89-14 | D4–D5 | No | | No | | 10 |
| | GP89-18 | D4–D5 | No | | No | | 10 |
| | GP89-23 | D4–D5 | No | | No | | 10 |

*Codes are for the Fifth Workshop unless preceded by (IV) which signifies the Fourth Workshop.
†A blank indicates that the mAb was not studied in the corresponding assay system. A compilation of mAb from the Fourth and Fifth Workshops and the recent literature is presented.
‡Literature references to mAb included in the Adhesion Structure Section of the Fifth Workshop are listed in Table 3 of the Section report [Springer *et al.*, AS1]. These references are provided for mAb not in the Fifth Workshop or if relevant data are included in a particular reference.
§Diamond and Springer, unpublished.

rhinovirus, and *Plasmodium falciparum*-infected erythrocytes to distinct, but in part overlapping sites in domain 1 [8–11]. The binding site for Mac-1 (CD11b/CD18) localizes to domain 3 [12,13]. The same strategy was employed to localize epitopes for many of the available CD54 mAb (Table 1) [8,10]. Two groups [8,10] have found that IgSF domains 1 and 2 appear conformationally linked because IgSF domain 1 cannot be expressed in the absence of IgSF domain 2. mAb with epitopes within IgSF domain 2 block binding of LFA-1 (Table 1).

Several participants in this Workshop confirmed the CD54 specificity of all submitted mAb by transfectant analysis [Craig *et al.*, AS4.5; Binnerts *et al.*, AS5.7; McDowall *et al.*, AS4.4; Klickstein *et al.*, AS4.8]. mAb epitopes were localized with IgSF domain chimeras [Craig *et al.*, AS4.5; McDowall *et al.*, AS4.4] and antibody cross-blocking experiments (Table 1) [Craig *et al.*, AS4.5; Klickstein *et al.*, AS4.8].

## Function

Functional studies from the current Workshop and the literature are summarized in Table 1. In keeping with the current model of CD54 function, a subset of mAb to IgSF domain 1 or 2, but not mAb to other domains, had significant ability to block binding

to LFA-1, *P. falciparum*-infected erythrocytes, and rhinoviruses.

## References


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# AS4.3 CD102 (ICAM-2) cluster report

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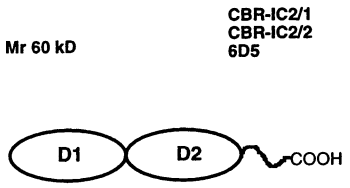
**CD102 (ICAM-2)**




The existence of a second ligand for LFA-1 was initially postulated based on the observation that some cell–cell interactions were blocked by monoclonal antibodies (mAb) to LFA-1, but not by mAb to ICAM-1. This activity was constitutively expressed on endothelium [1]. A cDNA for ICAM-2 was isolated from an endothelial library by screening for the ability of transfected COS cells to bind to LFA-1 [2]. deFougerolles *et al.* [3] obtained mAb to ICAM-2 expressed in COS-cell transfectants and Nortamo *et al.* [4] obtained mAb to ICAM-2 expressed in *Escherichia coli*. These mAb, submitted to the Fifth Workshop as S085 (CBR-IC2/1), S086 (CBR-IC2/2), and S099 (6D5), allowed ICAM-2 to be clustered as CD102. No ICAM-2 mAb were identified in the Endothelial Section [Klickstein *et al.*, E6.29] or in the Blind Panel [Shaw *et al.*, BP1.3].

## Cellular expression

Immunohistochemical and flow cytometric studies [Autschbach *et al.*, AS2.5; Koretz *et al.*, AS4.16; Krajewski *et al.*, AS10.10; unpublished Workshop studies by Athanasou, Bene, Cerf-Bensussan, Malizia, Patarroyo, Soligo, and Timens] found high levels of ICAM-2 on all endothelium and lower levels on a subset of haemopoietic cells, as previously published [3]. Interestingly, ICAM-2 is expressed on platelets as the sole ICAM, and can function as an LFA-1 ligand on these cells [5]. ICAM-2 was also found on thymic stromal cells [Friedrich *et al.*, AS6.15], but in general was not present on non-haemopoietic cells other than endothelium. Unlike ICAM-1, ICAM-2 is constitutively expressed and not responsive to lipopolysaccharide (LPS) or cytokines.