# AS4 Adhesion Structure Subpanel 4: CD50 (ICAM-3), CD54 (ICAM-1), and CD102 (ICAM-2)

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Subpanel 4 of the Adhesion Section was defined as the cellular adhesion molecules ('CAM') panel, and included monoclonal antibodies (mAb) designated by the submitter as recognizing ICAM-1 (CD54), ICAM-2 (CD102), ICAM-3 (CD50), or VCAM-1 (CD106). The 36 mAb of Subpanel 4 also included mAb to potentially functionally related molecules, PECAM-1 (CD31) on platelets and endothelium, CD43 on leucocytes, and the orphan mAb, 4A11, that recognizes an antigen on activated endothelium. Literature references for these mAb are listed in the Adhesion Structures Section report [Springer *et al.*, AS1, Table 3]. Prior to distribution to evaluators, the mAb of Subpanel 4 were pre-clustered by flow cytometric analysis of COS cells transfected with ICAM-1, -2, -3, or the seven-domain isoform of VCAM-1 to determine specificity. Sixty-five laboratories returned data, including 39 flow cytometric analyses, 12 immunohistochemical studies, 28 functional assays, and seven studies addressing mAb specificity with transfectants, purified proteins, or mAb cross-blocking. The specificity of each mAb was confirmed by analysis of transfected cells, or reaction with purified proteins for those recognizing CD50, CD54, CD102, and CD106, by at least two independent laboratories, while the CD43 mAb were shown to stain normal cells but not CD43-deficient cells (Table 1). Two laboratories examined Subpanel 4 mAb for cross-reacting mAb to swine [Kumagi *et al.*, A56.18; Howard and Sopp, unpublished Workshop report], cow [Howard and Sopp, unpublished Workshop report], or cynomologous monkey [Kumagi *et al.*, AS6.18].

Extensive epitope mapping was performed for both CD50 (ICAM-3) and CD54 (ICAM-1) with good agreement between different laboratories (Table 1). These mapping results, when correlated with the results of LFA-1 binding assays and *Plasmodium falciparum*-infected erythrocyte binding assays (Table 2), highlight an interesting distinction between these two otherwise quite similar molecules. Specifically, while only mAb to immunoglobulin superfamily (IgSF) domain 1 have inhibitory function in the case of CD50 (ICAM-3), mAb to both IgSF domain 1 and domain 2 have inhibitory function for CD54 (ICAM-1) (Tables 1 and 2).

Functional studies with mAb by participants in Subpanel 4 generally dealt with inhibition or stimulation of cell adhesion or with aggregation. Five laboratories returned data addressing lymphoid cell–stromal cell interactions together with flow cytometric analysis of antigen expression (Table 2) [Freedman *et al.*, B30.20; Dittel and LeBien, AS7/8.15; Olweus *et al.*, AS7/8.10; Lund-Johansen *et al.*, E6.13; Friedrich *et al.*, AS6.15; Behr *et al.*, AS6.12]. The stromal cells included lymphoid germinal centres, thymic stroma, and bone marrow stroma, and the lymphoid cells included $CD34^+$ cells and subsets thereof, and $CD10^+/IgM^-$ cells. When analysed together with results from Subpanel 5, there was general agreement that lymphoid VLA-4 (CD49d/CD29) binding to stromal VCAM-1 (CD106) was quantitatively most important, followed by lymphoid LFA-1 (CD11a/CD18) binding to stromal ICAM-1 (CD54).

Three laboratories (Table 2) [Ida *et al.*, AS5.8; Poignard *et al.*, unpublished Workshop report] reported data on inhibition of HIV-induced syncytia formation or of mAb antigen expression following transfection with HIV genes. For two of the three cell lines used, inhibition of syncytia formation was observed with a subset of CD50, CD54, and CD18 mAb, while, for the third cell line, CD54 mAb and not CD50 mAb were inhibitory. CD50 and CD11a antigen levels were increased upon transfection of Raji cells but not upon transfection of Jurkat cells with the HIV *Tat* gene.

In general, the imaginative and careful experiments performed in Subpanel 4 of the Adhesion Structure Section have extended our knowledge of CD50, CD54, and CD102 considerably. In the following chapters, a cluster report very briefly summarizes current knowledge about each CD and these cluster reports are followed by selected papers.

## Acknowledgements

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**Table 1** Specificities of Adhesion Structures Subpanel 4 mAb

| Workshop mAb | | | | | Specificity determined by | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Transfectant studies | | | Inhibition of binding to cells | |
| Code | Clone name | Donor | Species | Isotype | CD21 chimera, mutants*† | Ig chimeras, mutants†‡ | L-cell transfectants§ | S109 or S108¶ | S113, S114, or S115‖ |
| **CD50** | | | | | | | | | |
| S081 | BRIC79 | Anstee/Judson | Mouse | IgG2a | CD50, D1 | CD50, D1–D3 | CD50 | CD50, A | |
| S084 | CG106 | Cordell/Mason | Mouse | IgG | CD50, D1 | CD50, D1–D3 | CD50 | CD50, A | CD50, b |
| S087 | CBR-IC3/1 | de Fougerolles/ Springer | Mouse | IgG1 | CD50, D1 | CD50, D1–D3 | CD50 | CD50, A | CD50, b |
| S088 | CBR-IC3/2 | de Fougerolles/ Springer | Mouse | IgG2a | CD50, D2 | CD50, D1–D3 | CD50 | CD50, C | CD50, a |
| S089 | CBR-IC3/3 | de Fougerolles/ Springer | Mouse | IgG2a | CD50, D4–D5 | | CD50 | CD50, C | |
| S090 | CBR-IC3/4 | de Fougerolles/ Springer | Mouse | IgM | CD50, D4–D5 | | CD50 | | |
| S091 | CBR-IC3/5 | de Fougerolles/ Springer | Mouse | IgG2a | CD50, D4–D5 | | CD50 | CD50, C | |
| S092 | CBR-IC3/6 | de Fougerolles/ Springer | Mouse | IgE | CD50, D1 | CD50, D1–D3 | CD50 | CD50, A | CD50, b |
| S093 | WDS 3.A9 | de Smet | Mouse | IgG1 | CD50, D1 | CD50, D1–D3 | CD50 | | CD50, b |
| S106 | By44 | Pulford | Mouse | IgG | CD50, D1 | CD50, D1–D3 | CD50 | CD50, A | |
| S108 | TP1/24 | Sánchez-Madrid | Mouse | IgG2a | CD50, D2 | CD50, D1–D3 | CD50 | CD50, B | |
| S109 | HP2/19 | Sánchez-Madrid | Mouse | IgG2a | CD50, D1 | CD50, D1–D3 | CD50 | CD50, A | CD50, b |
| S112 | KS128 | Turley/Pulford/ Jones | Mouse | IgG1 | CD50, D1+D2 | CD50, D1–D3 | CD50 | CD50, C | CD50, c |
| S113 | 152-2D11 | Vilella | Mouse | IgG1 | CD50, D1+D2 | CD50, D1–D3 | CD50 | CD50, C | CD50, c |
| S114 | 140-11 | Vilella | Mouse | IgG2b | CD50, D1 | CD50, D1–D3 | CD50 | CD50, A | CD50, b |
| S115 | 101-1D2 | Vilella | Mouse | IgG1 | CD50, D2 | CD50, D1–D3 | CD50 | CD50, C | CD50, a |

| | | | | | Transfectant studies | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | CD21 chimera, point mutants*† | Ig chimeras, point mutants†‡ | L-cell transfectants§ | Various chimeras, point mutants**† |
| **CD54** | | | | | | | | |
| S082 | F10.2 | Bloem | Mouse | IgG1 | CD54 | CD54, D1 | CD54 | CD54, D1 |
| S083 | 1304.100.4 | Burmeister | Mouse | IgG2a | CD54 | | | CD54, D1 |

*(Continued)*

**Table 1** *(Continued)*

| | | | | | Transfectant studies | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | COS transfectant* | CHO cell transfectant†† | Flow cytometry‡‡ | |
| S094 | CBR-IC1/3 | Diamond/ Springer | Mouse | IgG2a | CD54 | CD54, D3–D5 | CD54 | CD54, D3–D4 |
| S095 | CBR-IC1/4 | Diamond/ Springer | Mouse | IgG1 | CD54 | CD54, D1 | | CD54, D1 |
| S096 | CBR-IC1/11 | Diamond/ Springer | Mouse | IgG1 | CD54 | CD54, D3–D5 | CD54 | CD54, D3–D4 |
| S097 | CBR-IC1/12 | Diamond/ Springer | Mouse | IgG1 | CD54 | CD54, D3–D5 | CD54 | CD54, D3–D4 |
| S098 | 7F7 | Dierich/Most | Mouse | IgG2a | CD54 | CD54, D1 | CD54 | CD54, D1 |
| S100 | 8-4A6 | Haskard | | | CD54 | CD54, D2 | CD54 | CD54, D2 |
| S105 | MAY.029 | Ohashi | Mouse | IgG1 | CD54 | CD54, D1 | | CD54, D1 |
| S107 | RR1/1 | Springer | Mouse | IgG1 | CD54 | CD54, D1 | CD54 | CD54, D1 |
| S116 | YH370 | A. Yamada | Mouse | IgG1 | CD54 | CD54, D1 | CD54 | CD54, D1 |
| **CD102** | | | | | | | | |
| S085 | CBR-IC2/1 | de Fougerolles/ Springer | Mouse | IgG2a | CD102 | | CD102 | |
| S086 | CBR-IC2/2 | de Fougerolles/ Springer | Mouse | IgG2a | CD102 | CD102 | CD102 | |
| S099 | 6D5 | Gahmberg | Mouse | IgG1 | CD102 | CD102 | | |
| **non-ICAM mAb** | | | | | | | | |
| S101 | E1/6 | Kawahara/ Bevilacqua | Mouse | IgG1 | CD106 | CD106 | | |
| S111 | HAE-2a | Tedder/Coulter | Mouse | IgG1 | CD106 | CD106 | | |
| S102 | 4A11 | Koch | Mouse | IgM | ? | | | |
| S110 | TP1/15 | Sánchez-Madrid | Mouse | IgG2 | CD31 | | | |
| S103 | G19-1 | Ledbetter | Mouse | IgG1 | | | CD43 | |
| S104 | G10-2 | Ledbetter | Mouse | IgG1 | | | CD43 | |

*Study by Klickstein's group using CD21 chimera and point mutants [Klickstein *et al.*, AS4.8] except for CD106 (VCAM-1) where a COS cell transfectant was used.
†The D number refers to the Ig superfamily domain containing the epitope. D4–D5 indicates the epitope is within domain 4 or 5, while D1 + D2 indicates that the mAb epitope requires both domains 1 and 2 or is located at the D1–D2 junction.
‡Study by Hogg's group using immunoglobulin chimeras and point mutants [McDowall *et al.*, AS4.4].
§Study by Figdor's group using L-cell transfectants [Binnerts *et al.*, AS5.7].
¶Study by Sánchez-Madrid's group of the inhibition of binding of $^{125}$I-labelled mAb S109 (HP2/19) or S108 (TP1/24) to cells; letter indicates epitope group [del Pozo *et al.*, AS4.10].
‖Study by Vilella's group of inhibition of the binding of fluorescein isothiocyanate (FITC)-labelled mAb S113 (152-2D11), S114 (140-11), or S115 (101-1D2) to cells; letter indicates epitope group [Vilella *et al.*, AS4.9].
**Transfectant study by Berendt's group using various chimeras [Craig *et al.*, AS4.5].
††Study by Bernard's group using a CHO cell transfectant.
‡‡CD43 specificity was determined by Klickstein's group using flow cytometry of normal and CD43-deficient cell lines.

**Table 2** Functional studies of Adhesion Structures Subpanel 4 mAb

| Workshop mAb | | % inhibition* of | | | | | Stimulation of homotypic aggregation | |
|---|---|---|---|---|---|---|---|---|
| Code | Clone name | PMA-stimulated U937 aggregation† | Activated T-cell adhesion to ICAM-expressing L cells‡ | HIV-induced MOLT-4 syncytia formation§ | Stimulated T-cell binding to ICAM-1 Fc chimera¶ | ~65% inhibition of primary MLR‖ | SKW3 cells** | T cells†† |
| **CD50** | | | | | | | | |
| S081 | BRIC79 | <10 | 70 | 79 | | + | + | − |
| S084 | CG106 | <10 | 90 | NT | | + | + | − |
| S087 | CBR-IC3/1 | <10 | 35 | 57 | | + | + + + | + + + + |
| S088 | CBR-IC3/2 | <10 | 35 | 52 | | 0 | 0 | − |
| S089 | CBR-IC3/3 | <10 | 35 | 44 | | 0 | 0 | − |
| S090 | CBR-IC3/4 | <10 | 45 | NT | | 0 | 0 | − |
| S091 | CBR-IC3/5 | <10 | 80 | 25 | | 0 | 0 | − |
| S092 | CBR-IC3/6 | CA | CA | 73 | | + | + + + | + + + + |
| S093 | WDS 3.A9 | <10 | 100 | NT | | + | 0 | − |
| S106 | By44 | <10 | 70 | NT | | + | + | + + + + |
| S108 | TP1/24 | <10 | 0 | NT | | 0 | 0 | − |
| S109 | HP2/19 | <10 | 0 | NT | | + | + + + | + + + + |
| S112 | KS128 | 0–50 | 0 | NT | | 0 | 0 | − |
| S113 | 152-2D11 | >90 | 0 | NT | | 0 | 0 | − |
| S114 | 140-11 | 50–100 | 95 | NT | | + | 0 | − |
| S115 | 101-1D2 | 50–100 | 100 | NT | | 0 | 0 | − |
| **CD54** | | | | | | | | |
| S082 | F10.2 | 100 | 50 | | 45 | | 0 | |
| S083 | 1304.100.4 | 100 | NT | | NT | | + | |
| S094 | CBR-IC1/3 | <10 | CA | | 10 | | 0 | |
| S095 | CBR-IC1/4 | 100 | NT | | 60 | | + | |
| S096 | CBR-IC1/11 | <10 | CA | | 0 | | 0 | |
| S097 | CBR-IC1/12 | 0–50 | 0 | | 0 | | 0 | |
| S098 | 7F7 | >90 | 0 | | 15 | | 0 | |
| S100 | 8-4A6 | 100 | 40 | | 40 | | + | |
| S105 | MAY.029 | 100 | NT | | 90 | | + | |
| S107 | RR1/1 | 100 | 100 | | 95 | | + | |
| S116 | YH370 | 100 | 100 | | 75 | | + | |
| **CD102** | | | | | | | | |
| S085 | CBR-IC2/1 | <10 | 0 | | | | | |
| S086 | CBR-IC2/2 | <10 | 95 | | | | | |
| S099 | 6D5 | 100 | NT | | | | | |

*CA, Cells aggregated; NT, not tested.
†Ikewaki' group: % inhibition of phorbol myristate acetate (PMA)-stimulated U937 aggregation.
‡Figdor's group: % inhibition of mAb NK1-L16-activated T-cell adhesion to L cells expressing ICAM-1, -2, or -3 [Binnerts *et al.*, AS5.7].
§Ida's group: % inhibition of HIV-induced MOLT-4 syncytia formation [Ida *et al.*, AS5.8].
¶Hogg's group: % inhibition of PMA-stimulated T cell binding to an ICAM-1 Fc chimera on plastic [McDowall *et al.*, AS4.4].
‖Vilella's group: approximately 65% inhibition of primary mixed lymphocyte reaction (MLR) [Vilella *et al.*, AS4.9].
**Study by deFougerolles and Klickstein (CD50) and Sattentau (CD54) of stimulation of homotypic aggregation of SKW3 cells [Poignard *et al.*, unpublished Workshop report].
††Bernard's group: stimulation of T-cell homotypic aggregation [Bernard *et al.*, AS4.12].