Published in final edited form as:

Cell Immunol. 2005 ; 236(1-2): 29–41. doi:10.1016/j.cellimm.2005.08.027.

Report on antibodies submitted to the stromal cell section of HLDA8

Christopher D. Buckley^{d,*}, Sapna Halder^d, Debbie Hardie^d, Garry Reynolds^d, Ruurd Torensma^a, Vanina Jodon De Villeroche^b, Daniele Brouty-Boye^b, and Clare M. Isacke^c

^aDepartment of Tumor immunology, NCMLS 187, UMC Nijmegen, PO Box 9001, 6500HB Nijmegen, The Netherlands ^bInserm U602, Hospital Paul Brousse, Villejuif, France ^cThe Breakthrough Breast Cancer Research Centre, Institute of Cancer Research, Chester Beatty Laboratories, 237 Fulham Road, London SW3 6JB, UK ^dMRC Center for Immune Regulation, Institute of Biomedical Research, University of Birmingham, Birmingham B15 2TT, UK

Abstract

The paradigm for tissue specific homing of leukocytes is the “area code” hypothesis, which predicts that a specific combination of adhesive interactions and chemokine signals from the endothelium directs leukocyte migration into specific tissue sites. This area code hypothesis has been supported by studies from previous HLDA workshops where endothelial specific cell antigens have been studied. Similarly, a clear haematopoietic “stem cell code” comprising the chemokine SDF-1 (CXCL12) and the adhesion receptor VCAM-1 (CD106) has been shown to contribute to the stem cell niche within bone marrow [1]. HLDA 7 included a section devoted to stem cell antigens, which began to define additional antigens important in these processes. During the course of HLDA 8 we have extended these observations to determine whether a more global stromal address code defined by fibroblasts, exists in variety of different tissues [2]. The stromal cell section in HLDA 8 was designed to complement the malignant cell, endothelial cell, and stem cell/progenitor cell sections. Seven new CD numbers were assigned to antibodies included in this section at the HLDA 8 Workshop meeting held during December 2004.

1. Introduction

1.1. What are stromal cells?

Two broad historical definitions of stromal cells exist. The first is that they are cells of mesenchymal origin that are non-epithelium, non-endothelium, and non-haematopoietic. The second is that they are the cells within the stroma and therefore include fibroblasts, endothelial cells, and tissue resident leukocytes. Within both these definitions, some stromal cells, particularly leukocytes and endothelial cells, have been covered by previous HLDA workshops. However, fibroblasts have not been previously covered and as a consequence it is these cells that have been the focus of the stromal cell section in HLDA8.

1.2. What is a fibroblast?

The most abundant cells of the stroma are fibroblasts, which are responsible for the synthesis and remodelling of extracellular matrix components. In addition, their ability to produce and respond to growth factors allows reciprocal interactions with other stromal cell

types, and with adjacent epithelial and endothelial structures. As a consequence, fibroblasts play a critical role during tissue development and homeostasis, and are often described as having a “sentinel” or “landscaping” function. Moreover, these functions contribute to the pathology of many diseases either directly for example by overproduction of matrix components during fibrosis and/or indirectly by influencing the behaviour of neighbouring cell types. In particular, it is now well recognized that fibroblasts and their secreted products are critical in tumour development and the progression of inflammatory diseases [3-7].

1.3. Not all fibroblasts are the same

Fibroblasts are a diverse cell type and display clear topographic differentiation and positional memory that is transcriptionally imprinted [8,9]. Despite this heterogeneity there are very few selective markers that allow clear discrimination between fibroblast subtypes. What is known is that fibroblasts are extremely versatile connective tissue cells and display a remarkable capacity to differentiate into other members of the connective tissue family including cartilage, bone, adipocyte, and smooth muscle cells. Further, not all fibroblasts are mesodermal in origin. For example, fibroblasts in the head and neck region are derived from the neural crest (ectoderm). Even within a single tissue there is growing evidence that fibroblasts are not a homogeneous population, but exist as subsets of cells, much like tissue macrophages and dendritic cells [10,11]. It is likely that connective tissue contains a mixture of distinct fibroblast lineages with mature fibroblasts existing side by side with immature fibroblasts (often called mesenchymal fibroblasts) that are capable of differentiating into other connective tissue cells. Tantalizing evidence now suggests that as is the case for endothelial cells, fibroblast precursors (termed fibrocytes) circulate in peripheral blood. These cells share many properties of bone marrow stromal stem cells and are capable of differentiating into several cell lineages. This diversity in phenotype and function that characterizes fibroblasts from different anatomical sites may play a significant role in the intrinsic susceptibility of different organs to inflammatory insults. It may also provide the molecular basis for the well described but as yet poorly understood clinical finding that relapses in chronic inflammation are often tissue and site specific. Furthermore, recent studies have shown that thymic fibroblasts play an important role in early T cell development, providing a molecular explanation for long standing observations that destruction of the cephalic neural crest results in retarded lymphoid development in the thymus, as well as producing craniofacial and cardiac defects [3].

A critical advance to this field will be to identify and characterize fibroblast subsets, and to determine the relative contribution of these different subsets to tissue homeostasis and disease progression in diseases such as chronic inflammation and cancer. Therefore, the aims of the stromal cell section in HLDA 8 were:

1. Identify fibroblast specific antibodies (or at least those with expression mainly limited to fibroblasts).
2. Identify antibodies that can discriminate between fibroblasts from different sites.
3. Identify antibodies that can discriminate between normal tissue fibroblasts from one site and diseased tissue (fibroblasts) from the same site.

2. Methods

Antibodies were solicited from scientists in the field as well as open recruitment during the period January 2001 to June 2004. This resulted in 62 potential antibodies being assigned to the HLDA8 stromal cell section. These 62 antibodies were screened by immunohistochemistry of frozen sections of tonsil, synovium, liver, and skin as well as by flow cytometry of peripheral blood and tonsil lymphocytes. The aim of the screen was to

identify those antibodies that recognized epitopes that were relatively restricted to stromal cells (endothelium, epithelium, and fibroblasts) and were not expressed on leucocytes. In some cases where expression was confined to monocytes (CD14 positive) and/or tissue infiltrating leucocytes and not any other peripheral blood leucocytes, these antibodies were considered for further evaluation as there is now accumulating evidence that some fibroblasts can be derived from a circulating peripheral blood monocytes [12]. From the 62 antibodies submitted (Table 1), 18 antibodies plus 2 extra antibodies 80324 and 80448 from the stem cell section of HLDA 8, were selected and subjected to further screens by three independent laboratories in the United Kingdom (CDB, SH, DH, GR, CMI) Netherlands (RT), and France (V JDV, DB-B). These additional screens were designed to (a) determine their subcellular localization and their differential distribution on primary fibroblasts isolated from different tissues (bone marrow, spleen, liver synovium, lung) (b) determine whether the antigens recognized by these antibodies were cation and protease sensitive. Cation and protease sensitivity add biochemical data (for example cell surface integrins have cation sensitive epitopes), and protease sensitivity helps to confirm that the epitope recognized by the antibody is a protein.

3. Results

An example of how the data was collected for each antibody in the primary screen is shown for antibody 80167 (E-Cadherin) in Fig. 1. Of the 20 antibodies that passed the first screen (relatively restricted expression on stromal cells) three were found to have epitopes expressed on peripheral blood leucocytes and were then identified to recognize the previously designated CD antigens CD13 (80274), CD44 (80394), and CD166 (80273) Fig. 2. One of the antibodies was demonstrated to be against an intracellular enzyme prostaglandin synthase (80393) Fig. 3. Seven antibodies were assigned new CD numbers at the workshop based on the fact that the antigen had been cloned and did not already have a CD number (80167, 80168, 80324, 80392, 80448, 80646, 80645). Of the remaining nine antibodies, insufficient information (the antigen was not cloned and there was no molecular or biochemical data available) precluded the assignment of CD numbers at this workshop (80169, 80170, 80171, 80172, 80202, 80213, 80275, 80276, 80277). Examples of three of these non-designated antibodies (80169), (80170), (80202) are shown in Fig. 4. In the secondary screens, no antibody was found that could clearly discriminate between subsets of fibroblasts (i.e., had distinct biphasic distribution on flow cytometric analysis of primary fibroblasts) or between fibroblasts isolated from different sites (data not shown). However, there were clear differences between antibodies that recognized extracellular epitopes as well as differences between antibodies in terms of cation and protease sensitivity (summarized in Table 2).

4. Conclusions

The low number of antibodies analyzed in this section was insufficient to obtain an overall description of fibroblast cell surface antigens. However from the antibodies that were studied it was notable that (a) although antibodies were characterized as recognizing antigens that were predominantly expressed by fibroblasts, no fibroblast specific antibodies were identified, (b) no antibodies were identified that discriminated between fibroblasts from different anatomical sites. It remains to be determined whether the antibodies that have been characterized discriminate between fibroblasts from diseased and normal tissue [13].

The relatively low number of antibodies submitted to the stromal cell section reflects two important issues. First, that classification of fibroblast cell surface proteins remains an immature field. Second, workers in the field have increasingly employed transcriptional profiling to characterize fibroblasts and other stromal cells rather than an antibody based

approach (for examples see Refs. [8,9]). However, the problem with the latter approach is that antibodies to some of the proteins identified using transcriptional profiling are frequently not available to validate results and to further analyze antigen distribution. Furthermore, transcriptional profiling does not permit the isolation and further functional characterization of fibroblast isolated ex vivo. As a consequence, it is our opinion that an antibody based approach to characterizing fibroblast cell surface proteins is still a valid and valuable one. Moreover with increasing interest in the role of stromal cells such as fibroblasts in disease progression (for example cancer and chronic inflammation), we would encourage others in the field to continue to generate antibodies and characterize stromal cell surface antigens so that these reagents can form the basis of further HLDA workshops.

References

- [1]. Tokoyoda K, Egawa T, Sugiyama T, Choi BI, Nagasawa T. Cellular niches controlling B lymphocyte behaviour within bone marrow during development. *Immunity*. 2004; 20:707–718. [PubMed: 15189736]
- [2]. Parsonage G, Filer AD, Haworth O, Nash GB, Rainger GE, Salmon M, Buckley CD. A stromal area postcode defined by fibroblasts. *Trends Immunol.* 2005; 26:150–156. [PubMed: 15745857]
- [3]. Buckley CD, Pilling D, Lord JM, Akbar AN, Scheel-Toellner D, Salmon M. Fibroblasts regulate the switch from acute resolving to chronic persistent inflammation. *Trends Immunol.* 2001; 22:199–204. [PubMed: 11274925]
- [4]. Elenbaas B, Weinberg RA. Heterotypic signaling between epithelial tumor cells and fibroblasts in carcinoma formation. *Exp. Cell Res.* 2001; 264:169–184. [PubMed: 11237532]
- [5]. Tlsty TD. Stromal cells can contribute oncogenic signals. *Semin. Cancer Biol.* 2001; 11:97–104. [PubMed: 11322829]
- [6]. De Wever O, Mareel M. Role of tissue stroma in cancer cell invasion. *J. Pathol.* 2003; 200:429–447. [PubMed: 12845611]
- [7]. Silzle T, Randolph GJ, Kreutz M, Kunz-Schughart LA. The fibroblast: sentinel cell and local immune modulator in tumor tissue. *Int. J. Cancer.* 2004; 108:173–180. [PubMed: 14639599]
- [8]. Chang HY, Chi JT, Dudoit S, Bondre C, van de Rijn M, Botstein D, Brown PO. Diversity, topographic differentiation, and positional memory in human fibroblasts. *Proc. Natl. Acad. Sci. USA.* 2002; 99:12877–12882. [PubMed: 12297622]
- [9]. Parsonage G, Falciani F, Burman A, Filer A, Ross E, Bofill M, Martin S, Salmon M, Buckley CD. Global gene expression profiles in fibroblasts from synovial skin and lymphoid tissue reveals distinct cytokine and chemokine expression patterns. *Thromb. Haemost.* 2003; 90:688–697. [PubMed: 14515190]
- [10]. Postlethwaite AE, Shigemitsu H, Kanagat S. Cellular origins of fibroblasts: possible implications for organ fibrosis in systemic sclerosis. *Curr. Opin. Rheumatol.* 16(2004):733–738. [PubMed: 15577612]
- [11]. Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. *Nature.* 2004; 432:332–337. [PubMed: 15549095]
- [12]. Pilling D, Buckley CD, Salmon M, Gomer RH. Inhibition of fibrocyte differentiation by serum amyloid P. *J. Immunol.* 2003; 171:5537–5546. [PubMed: 14607961]
- [13]. MacFadyen JR, Haworth O, Robertson D, Hardie D, Webster M-T, Morris HR, Panico M, Sutton-Smith M, Dell A, van der Geer P, Wienke D, Buckley CD, Isacke CM. Endosialin (TEM1, CD248) is a marker of stromal fibroblasts and is not selectively expressed on tumour endothelium. *FEBS Lett.* 2005; 579:2569–2575. [PubMed: 15862292]
- [14]. Li L, Yoon SO, Fu DD, Zhang X, Choi YS. Novel follicular dendritic cell molecule, 8D6, collaborates with CD44 in supporting lymphomagenesis by a Burkitt lymphoma cell lines, L3055. *Blood.* 2004; 104:815–821. [PubMed: 15090445]
- [15]. Lee IY, Choe J. Human follicular dendritic cells and fibroblasts share the 3C8 antigen. *Biochem. Biophys. Res. Commun.* 2003; 304:701–707. [PubMed: 12727211]
- [16]. Buhring J-J, Muller T, Herbst R, Cole S, Rappold I, Schuller W, Zhu X, Fritzsche U, Faul C, Armeanu S, Ullrich A, Klein G, Schmidt H. The adhesion molecule E-cadherin and a surface

- antigen recognized by the antibody 9C4 are selectively expressed on erythroid cells of defined maturational stages. *Leukemia*. 1996; 10:106–116. [PubMed: 8558914]
- [17]. Lammers R, Giesert C, Grünebach F, Marxer A, Vogel W, Bühring H-J. Monoclonal antibody 9C4 recognizes epithelial cellular adhesion molecule, a cell surface antigen expressed in early steps of erythropoiesis. *Exp. Hematol*. 2002; 30:537–545. [PubMed: 12063020]
- [18]. Sheikh H, Yarwood H, Ashworth A, Isacke CM. Endo180, an endocytic recycling glycoprotein related to the macrophage mannose receptor is expressed on fibroblasts, endothelial cells and macrophages and functions as a lectin receptor. *J. Cell Sci*. 2000; 113:1021–1032. [PubMed: 10683150]
- [19]. Li L, Zhang X, Kovacic S, Long AJ, Bourque K, Wood CR, Choi YS. Identification of a human follicular dendritic cell molecule that stimulates germinal centre B cell growth. *J. Exp. Med*. 2000; 191:1077–1083. [PubMed: 10727470]

Fig. 1. Example of data accumulated during primary screen on all 62 antibodies submitted. Information in the spreadsheet format includes data from immunohistochemistry screens on tonsil and liver sections (green is the test antibody and red is nuclear counter stain) as well as screens on leucocytes isolated from tonsil and peripheral blood. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

Fig. 2.
Examples of three antibodies which recognized non-stromal cells and were subsequently found 10 recognize CD13 (A), CD44 (B), and CD166 (C). In the immunofluorescence pictures, green is the test antibody and red is the nuclear counterstain. (see Ref. [14] for more information on (B)). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

Fig. 3.
Example of an antibody that appeared to be stromal specific but when cloned was found to identify the intracellular enzyme prostaglandin synthase ([15] and personal communication). In the immunofluorescence pictures, green is the test antibody and red is the nuclear counterstain. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

Fig. 4. Examples of three of the nine antibodies that remain unclassified and did not receive CD numbers at the workshop. (A) 80169, (B) 80170, and (C) 80202. In the immunofluorescence pictures, green is the test antibody and red is the nuclear counterstain. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

Table 1

Details of the 62 antibodies submitted to the stromal section of HLDA 8

Workshop number	Antibody	Isotype	Immunogen	Specificity
80100	MIMA-24	IgG1	Plasmid cDNA/transfected 293T cells	Kell protein
80101	MIMA-52	IgG2a	Plasmid cDNA/transfected 293T cells	Dombrock protein ART4
80102	MIMA-53	IgG2a	Plasmid cDNA/transfected 293T cells	Dombrock protein ART4
80103	MIMA-46	IgM	Transfected 293T cells	I-like
80104	MIMA-51	IgG2b	DTT-treated hRBCs	Rh:17
80105	NBL-1	IgG1	Peptides	Xg(a)
80106	BANR S10	IgA	Hu erythrocytes (male)	CD99
80107	MSG-B1	IgG2b	Synthetic peptides	CD99
80111	ZCH-4D5	IgG1	KG1a cell line	?CD45
80114	Y129	IgG2a	Human myeloma cell line, KPC-32	Human natural HM 1.24
80465	RS38E	IgG1	Hu BST-2 expressed BALB 3T3 cells	Human BST-2
80466	K5C1	IgG1	Recombinant human HM1.24	Human recombinant HM1.24
80117	HUTS-21	IgG2a	Purified $\beta 1$ integrins	CD29
80142	1-456	IgG1	Myeloid derived-DC	CD58
80143	5-341	IgG2a	Myeloid derived-DC	CD54
80160	N6 B6	IgG2a	Nalm-1	CD164
80162	67 D2	IgG1	T-47D breast tumour cells	CD164
80163	B1 D5	IgG2a	SIRP $\beta 1$ transfected NIH-3T3 cells	CD172b
80164	B4 B6	IgG1	SIRP $\beta 1$ transfected NIH-3T3 cells	CD172b
80167	67 A4	IgG1	T-47D breast tumour cells	E-cadherin
80168	9 C4	IgG2b	DU-4475 breast tumour cell line	Ep-CAM
80169	57 D2	IgG1	TF-1 erythroleukemia cell line	Unknown
80170	W4A5			Subsets of mesenchymal stem cells +neuronal progenitor cells
80171	W8B2B10			Subsets of mesenchymal stem cells
80172	W8C3B3			Subsets of mesenchymal stem cells
80201	W1B9	IgM	Patient derived (no immunization)	RBC membrane
80202	3A11	IgG1	HLB-100 cells (human)	Novel CD6 ligand
80203	ZCH-2G7	IgG1	Fresh TdT + AML blasts	Unknown CD, non-lineage
80204	ZCH-5E2	IgG2b	KG1a cell line	Unknown CD, non-lineage
80205	ZCH-4B6	IgG1	KG1a cell line	Unknown CD, non-lineage
80206	ZCH-8D1a	IgG2a	KG1a cell line	Unknown CD, non-lineage
80207	ZCH-9C7	IgM	KG1a cell line	Unknown CD, non-lineage
80208	ZCH-2H7b	IgG1	KG1a cell line	Unknown CD, non-lineage
80209	ZCH-3B8	IgG1	KG1a cell line	Unknown CD, non-lineage
80210	ZCH-4E3	IgG1	KG1a cell line	Unknown CD, non-lineage
80211	ZCH-3B12	IgG1	KG1a cell line	Unknown CD, non-lineage
80212	ZCH-5H8	IgG2b	KG1a cell line	Unknown CD, non-lineage
80213	ZCH-2B8a	IgG2a	KG1a cell line	Unknown CD, non-lineage
80214	ZCH-2F10	IgG1	Fresh TdT + AML blasts	Unknown CD, non-lineage

Workshop number	Antibody	Isotype	Immunogen	Specificity
80226	ZCH-2D3	IgG1	Fresh TdT + AML blasts	Unknown CD, non-lineage
80268	B-L28			?CD58
80270	B-D15			?CD29
80273	A1		Hu BM derived stromal cells	Bone marrow derived stromal
80274	A8		Hu BM derived stromal cells	Bone marrow derived stromal
80275	A2		Hu BM derived stromal cells	Bone marrow derived stromal
80276	E5		Hu BM derived stromal cells	Bone marrow derived stromal
80277	E6		Hu BM derived stromal cells	Bone marrow derived stromal
80310	FB21	IgM	Hu lymphoid cell line	
80314	CUB 1			?CDCP1
80315	CUB 2			?CDCP1
80317	CUB 4			?CDCP1
80337	P1B5	IgG1, k	HT 1080 fibrosarcoma cells	α 3 subunit of integrin α 3 β 1
80338	P4G9	IgG3, k	Jurkat T lymphoblasts	α 4 subunit of integrin α 4 β 1
80339	P1D6	IgG3, k	Lymphokine activated killer cells	α 5 subunit of integrin α 5 β 1
80359	NCH-38	IgG1, k	E-cadherin (uvomorulin)	120kDa of mature E-cadherin + 82kDa fragment of E-cadherin
80392	8D6	IgG1,k		Follicular dendritic cells
80393	3C8			Follicular dendritic cells
80394	4G10			Follicular dendritic cells
80437	11G5a			CD151
80462	188331		EC domain of human jagged-1 (aa I-296)	Human jagged-1
80464	87908		EC domain of human BMPR-1 α	Human BMPR-1 α
80465	88614		EC domain of human BST-2	Human BMPR-1 β

Table 2

Details of the 20 antibodies that passed the first screen and were then analyzed for expression on a range of primary fibroblasts as well as cation sensitivity, protease resistance, and intracellular or extracellular expression

Workshop number	Antibody	Isotype	Immunogen	Specificity	Cation sensitive	Trypsin sensitive	Extracellular or intracellular	CD number
80167	67 A4	IgG1	T-47D breast tumour cells	E-cadherin [16]	No	No	Extracellular	CD324
80168	9 C4	IgG2b	DU-4475 breast tumour cell line	Ep-CAM [17]	No	No	Extracellular	CD326
80169	57 D2	IgG1	TF-1 erythroleukemia cell line	Unknown	?	?	?	-
80170	W4A5	IgG1	WER1-RB-1 retinoblastoma cell line	Subsets of mesenchymal stem cells + neuronal progenitor cells	No	No	extracellular	-
80171	W8B2B10	IgG1	WER1-RB-1 retinoblastoma cell line	Subsets of mesenchymal stem cells	No	No	Extracellular	-
80172	W8C3B3	IgG1	WER1-RB-1 retinoblastoma cell line	Subsets of mesenchymal stem cells	?	?	Extracellular	-
80202	3A11	IgG1	HBL-100 cells (human)	Novel CD6 ligand	No	Yes	Extracellular	-
80213	ZCH-2B8a	IgG2a	KG1a cell line	Unknown CD, non-lineage	?	?	?	-
80273	A1		Hu BM derived stromal cells	CD166 (pers. comm.)	No	No	Extracellular	-
80274	A8		Hu BM derived stromal cells	CD13 (pers. comm.)	No	No	Extracellular	-
80275	A2		Hu BM derived stromal cells	Bone marrow derived stromal	Yes	No	Extracellular	-
80276	E5		Hu BM derived stromal cells	Bone marrow derived stromal	No	No	?	-
80277	E6		Hu BM derived stromal cells	Bone marrow derived stromal	No	No	Extracellular	-
80324	E1/183	IgG1	AG1523 human fibroblasts	Endo180/uPARAP [18]	?	No	Extracellular	CD280
80392	8D6		Follicular dendritic cells	8D6 transmembrane antigen [19]	No	No	Extracellular	CD320
80393	3C8		Follicular dendritic cells	Prostaglandin synthase ([15]; and pers. comm.)	No	No	Intracellular	-
80394	4G10		Follicular dendritic cells	CD44 [14]	No	No	Extracellular	-
80448	B1/473	IgG1	AG1523 human fibroblasts	Endosialin ([13])	?	Yes	Extracellular	CD248
80464	87908	IgG2b	EC domain of hu BMPR-1a	Human BMPR-1a	No	No	Extracellular	CD292
80645	RS38E	IgG1	Hu BST-2 expressed in 3T3 cells	Human BST-2	?	?	?	CD317