Virchows Arch (1998) 432:343–347 © Springer-Verlag 1998

ORIGINAL ARTICLE

Ulrike Seitzer · Dagmar Scheel-Toellner
Taila Mattern · Helmut Haas · Hans-Dieter Flad
Johannes Gerdes

Staining pattern of seven monoclonal anti-CD26 antibodies in leprosy: implications for the use of CD26 as a surrogate marker of a human Th1-like reaction

Received: 14 August 1997 / Accepted: 7 November 1997

Abstract In a previous study using the monoclonal anti-CD26 antibody MIB-DS2/7 in leprosy and other granulomatous diseases, it was shown that CD26 may be a candidate for use as an operational marker of a human Th1-like reaction. In this follow-up study, we compared seven different monoclonal anti-CD26 antibodies with respect to their staining pattern in lepromatous and tuberculoid leprosy tissues. Three distinct staining patterns became apparent in this anti-CD26 antibody panel: staining of T-lymphocytes and of connective tissue; staining of T-lymphocytes, connective tissue and macrophages; and almost no staining of T-lymphocytes but staining of connective tissue and macrophages. The two antibodies assigned to the first staining pattern, including MIB-DS2/7, were found to be most suitable for the operational discrimination between Th1-like and Th2-like reactions in leprosy. The antibodies assigned to staining patterns 2 and 3 did not allow this discrimination. Although all seven monoclonal antibodies investigated were specific for CD26, only two were found to be useful in identifying a Th1-like immune reaction in human tissue.

Key words Th1 · Dipeptidylpeptidase IV · Immunoenzymatic staining · MIB-DS2/7

Introduction

T helper lymphocytes can be divided into two distinct subsets based on their cytokine profile. The functionally different subsets of T helper 1 (Th1) and Th2 cells were originally described in mouse CD4+ T cells [16] and later in human T cells [20]. The Th1 subset secretes cytokines usually associated with inflammation, such as interleukin-2 and interferon-γ (IFNγ) and induces cell-mediated immune responses. The Th2 subset produces cytokines such as interleukin-4 and interleukin-5, which help B cells to proliferate and differentiate, and is associated with humoral-type responses [15]. Because their respective cytokines act antagonistically, these two cell populations mutually regulate their functions [24], which may explain a strong bias toward either a Th1 or a Th2 response during many infections in mice and humans [17]. The differentiation of Th precursor cells into Th1 or Th2 cells has important implications in terms of susceptibility or resistance to particular diseases. For instance, in *Leishmania major* parasitic infections in mice there is reciprocal expression of IFNγ and interleukin-4 in mice of different backgrounds, which correlates with resolution or progression of the disease [9]. In human immunodeficiency virus-infected individuals a switch from a Th1 to a Th2 phenotype occurs as symptoms worsen [2]. Because of the pathophysiological relevance of these T-cell subsets, an immunophenotypical marker allowing the discrimination of these cells in immunohistology or flow cytometric analysis would be of great interest. The monoclonal antibody MIB-DS2/7 was generated by immunizing mice with a human Th1-like T-cell clone and was selected after screening owing to a high reactivity with different Th1-like clones and a moderate reactivity with Th2-like clones. Biochemical characterization of the antibody revealed it to be specific for dipeptidyl peptidase IV (DP IV, CD26) [22]. Using this antibody, a three-colour immunofluorescence staining for CD4, CD45RO and CD26 resulted in the enrichment of Th1-like cells as defined by their cytokine mRNA expression [21]. To see whether the strong reactivity of MIB-DS2/7 with Th1-like T-cell clones is reflected in pathological conditions ex vivo, immunohistological investigations were performed on tissues affected by granulomatous inflammatory reactions, including leprosy, sarcoidosis and Piringer's lymphadenitis [23]. In leprosy, the clinical forms constitute a spectrum that closely correlates with the de-

U. Seitzer · T. Mattern · H.-D. Flad · J. Gerdes (✉)
Department of Immunology and Cell Biology,
Forschungszentrum Borstel, Parkallee 22,
D-23845 Borstel, Germany
Tel.: (+49) 4537-188424, Fax: (+49) 4537-188404

H. Haas
Department of Clinical Medicine,
Forschungszentrum Borstel, Germany

D. Scheel-Toellner
Department of Rheumatology, University of Birmingham, UK

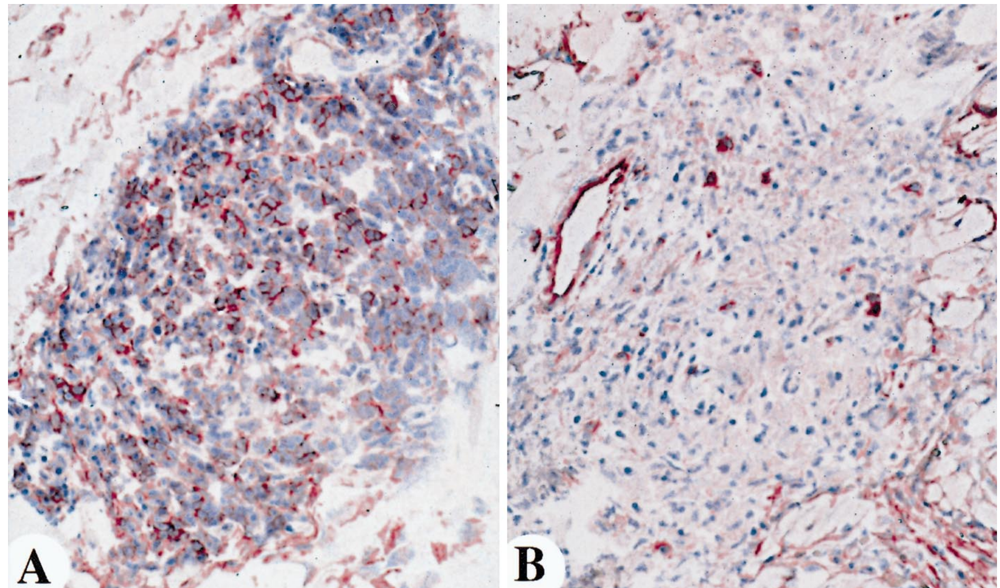

Fig. 1 Representative immunohistological assessment of CD26-positive T-cells in **A** a case of tuberculoid leprosy (Th1-like) and **B** a case of lepromatous leprosy (Th2-like) using MIB-DS2/7. In tuberculoid leprosy, a high incidence of CD26-expressing T-cells was detected in the granuloma. In lepromatous leprosy, only a very few CD26-positive cells could be discerned. Original magnification 50×

Table 1 Monoclonal anti-CD26 antibodies used in this study

Clone	Isotype	Antigen	Originator	Commercial source
MIB-DS2/7	G1k	Human Th1 clone	[23]	Dianova
4EL-1C7 (Ta1)	G1	IL-2 dependent human T-cell line EL156	[6]	Coulter
202–36	G2b	Human T-cell line	R. Vilella	–
EF5/A3	G1	CD26-positive U937	[11, 18]	–
L272	G2a	IL-2 dependent human CTL line		Becton Dickinson
BA5	G2ak	Human T cell clone		Immunotech
2A6	G1k	Renal cell adeno carcinoma cell line	S. Poppema	–

gree of cellular immunity [15], tuberculoid leprosy representing one extreme of the spectrum, with a primary Th1-like reaction, and lepromatous leprosy at the other extreme, with a predominantly Th2-like reaction [29]. The investigations in leprosy showed strong signals for IFNγ and CD26 in all cases of tuberculoid leprosy investigated, in contrast to no or very weak signals in lepromatous leprosy. Immunofluorescence double-labelling also demonstrated the coexpression of CD26 and IFNγ by identical cell populations [23]. In summary, these investigations suggest that a high expression of CD26, as detected by MIB-DS2/7, is indicative of a Th1-like immune reaction and that MIB-DS2/7 may be used as an operational marker of a human Th1-like reaction (Fig. 1). These observations were confirmed by the investigations of Willheim et al., who demonstrated a correlation between high CD26 cell surface expression and the production of Th1-like cytokines by T-cell clones [28].

It has been observed that different anti-CD26 antibodies detect varying amounts of CD26 expression on resting and activated T-cells [6, 14, 21, 25] and also elicit differing effects in in vitro systems for studying stimulation of T-cells [4, 27]. In addition, up to 11 different immunoreactive molecular forms of CD26 were discernible in cell lysates of activated lymphocytes [12]. Given these observations, we investigated and compared seven different anti-CD26 antibodies with respect to their staining pattern in lepromatous and tuberculoid leprosy skin biopsies in order to determine their suitability as potential surrogate markers of a human Th1-like reaction.

Materials and methods

Skin biopsies were obtained from patients suffering from leprosy and taking part in the leprosy eradication programme conducted by the Department of Leprosy of the Ministry of Health and Welfare, Paraguay. Patients were classified according to the clinicopathological criteria of Ridley and Jopling [19]. All biopsies were taken from untreated patients; they were snap-frozen in liquid nitrogen and stored until use at –80°C.

Cryostat frozen sections were fixed in acetone for 30 min, followed by fixation in chloroform for 30 min. After fixation the leprosy sections were preincubated with rabbit normal serum for 30 min to block nonspecific binding. Incubation with the primary antibody was performed for 30 min, and immunostaining was undertaken according to the APAAP (alkaline phosphatase anti-alkaline phosphatase) method with New Fuchsin development [3]. Finally, slides were counterstained with haematoxylin and mounted. Immunostaining was controlled by the use of only the secondary reagents and isotype controls to confirm their specificity.

A description of the antibodies used in this study is given in Table 1. All anti-CD26 antibodies except MIB-DS2/7 [23] and Ta1 [1] were kindly provided by the non-lineage section of the VIth International Workshop and Conference on Human Leukocyte Differentiation Antigens, Kobe, Japan, 1996, and were described as specific for CD26 [7, 8]. The MIB-DS2/7 antibody is available from Dianova (Hamburg, Germany) and will be freely distributed by the authors on request.

Table 2 Staining intensities and patterns[a] of seven different anti-CD26 antibodies on frozen sections of normal kidney, hyperplastic tonsil, and lepromatous and tuberculoid leprosy skin biopsies (+ to ++++ degrees of positive staining, (+) faint staining, – no staining, *LL* lepromatous leprosy, *TL* tuberculoid leprosy, *C* connective tissue, *M* macrophages, *L* lymphocytes)

Antibody	Kidney	Tonsil	LL (*n*=7)	TL (*n*=7)	Staining pattern
MIB DS2/7	++++	C ++ M (+) L +++	C ++ M (+) L +	C +++ M (+) L ++++	1
2A6	++++	C ++ M – L ++++	C ++ M – L ++	C ++ M – L ++++	1
EF5/A3	++++	C + M + L + C (+)	C ++ M (+) L + C ++	C ++ M + L +++ C +	1–2
L272	++++	M + L ++ C –	M ++ L + C ++	M ++ L +++ C +	2
Ta1	++++	M (+) L + C –	M + L + C (+)	M ++ L ++ C (+)	2
202–36	++++	M (+) L – C –	M + L (+) C +	M + L + C +	3
BA5	++++	M (+) L –	M ++ L +	M ++ L +	3

[a]*Staining patterns*
1 staining of lymphocytes and connective tissue,
2 staining of lymphocytes, connective tissue and macrophages,
3: staining of connective tissue, macrophages and virtually no lymphocytes

Results

The results of the staining are summarized in Table 2. Serial staining of frozen sections of human kidney revealed equal intensity and distribution of staining of proximal tubular epithelium with all antibodies. The localization of dipeptidyl peptidase IV in this tissue has been described [25], and it can thus be used as a CD26-positive control. In marked contrast to this very uniform staining, substantial differences between antibodies were seen in staining intensity and pattern of serial frozen sections of hyperplastic human tonsils, which were used as normal lymphoid tissue controls. The differences were most prominent in the staining of T-lymphocytes, ranging from strong staining (MIB-DS2/7, 2A6) to no staining at all (202–36, BA5). The situation in *Mycobacterium leprae*-infected tissue was similarly remarkably diverse. The staining of seven different lepromatous and tuberculoid skin biopsy samples was averaged to give an individual staining pattern of lepromatous and tuberculoid tissue for each antibody included in the study. The staining pattern was derived primarily by evaluating the amount and intensity of positively stained T-lymphocytes as the most important variables. The staining intensity of connective tissue and macrophages was included in the staining pattern to evaluate the specificity of the staining for T-lymphocytes. The inclusion of these criteria in the evaluation of the staining pattern resulted in three distinct patterns to which the anti-CD26 antibodies could be attributed: staining of T-lymphocytes and connective tissue; staining of T-lymphocytes, connective tissue and macrophages; almost no staining of T-lymphocytes, but staining of connective tissue and macrophages. The antibodies MIB-DS2/7 and 2A6, both assigned to the first staining pattern, showed a greatly increased reactivity with T-cells in the tuberculoid lesions compared with the lepromatous lesions. Staining of connective tissue and very faint staining of macrophages did not hinder the observation of this distinction. Typical staining for CD26, as seen with MIB-DS2/7 in tuberculoid and lepromatous leprosy, is shown in Fig. 1. The antibodies EF5/A3, L272 and Ta1, assigned to the second staining pattern, also showed higher reactivity with T-cells in the tuberculoid lesions than in the lepromatous lesions. However, in this group staining of macrophages was also significantly increased, thus making a distinction between tuberculoid and lepromatous leprosy difficult. The remaining antibodies (202-36, BA5), which were classed as having the third staining pattern, were found to show very weak staining of only a small number of T-lymphocytes and no noticeably more intense staining of T-cells could be discerned in the tuberculoid granulomas than in the lepromatous granulomas. In addition, staining of macrophages was significantly increased, to the extent that a distinction between tuberculoid and lepromatous tissue was virtually impossible.

Discussion

The seven different anti-CD26 antibodies used in this study show striking differences in their staining pattern of T-cells in normal lymphoid and in the granulomas of *M. leprae*-infected tissue. This is somewhat difficult to explain considering that the staining pattern in normal human kidney was the same for all antibodies, which also proved that the antibodies were being applied at optimal dilutions. However, the description of multiple immunoreactive isoforms of CD26 [12] and splice variants of CD26 mRNA in activated T-cells [26], and also the

finding of varying amounts of CD26 detected on resting and activated T-cells by different anti-CD26 antibodies [6, 14, 21, 25] lend credence to the differences observed in this study. There are many possible explanations for these variations; most are highly speculative, such as the diversity of the antigens used to generate the antibodies (Table 1), the specific recognition of differentially glycosylated forms of CD26 [13], or even cross reactivity with the recently described novel forms of proteins similar to CD26 [5, 10].

CD26 is a widespread molecule and is found in a variety of cells besides T-cells, including fibroblasts and macrophages [26]. Since staining of these three cell types was evident in our study, all three were included in the evaluation of the staining pattern for the antibodies. The differences in the staining pattern of T-lymphocytes played the most prominent part in evaluation of the antibodies for suitability as operational markers of a human Th1-like reaction, as described for MIB-DS2/7 [21–23]. Staining of connective tissue (fibroblasts) was not found to obstruct the evaluation of the staining of T-lymphocytes, whereas strong staining of macrophages greatly hindered the assessment of the stained T-lymphocytes fraction in the tissue. According to these criteria, only two of the seven antibodies used, MIB-DS2/7 and 2A6, were found to be suitable for utilization as operational markers of a Th1-like reaction, showing markedly more intense staining of T-lymphocytes in tuberculoid leprosy granulomas (Th1-like) than in lepromatous leprosy granulomas (Th2-like). Since these two antibodies also showed much more pronounced staining of T-lymphocytes in normal lymphoid tissue and since the lack of CD26-positive cells in lepromatous leprosy cannot be explained by the absence either of T cells or of the CD4- or CD8-positive subset [23], these two antibodies must recognize an isoform or molecular form of CD26 that is appropriate for operational Th1-like discrimination in diseased tissue.

This study confirms and further corroborates the previous finding that expression of CD26 can be used as an operational marker of a Th1-like reaction in human tissue. However, restrictions have to be made concerning the detection of CD26, since seven different monoclonal antibodies specific for CD26 showed a wide range of reactivity patterns with normal lymphoid and granulomatous tissue. Thus, only two of the seven anti-CD26 antibodies were found to be useful in discriminating between a Th1- and Th2-like immune reaction in established tuberculoid and lepromatous leprosy.

Acknowledgements The expert technical assistance of Ms. M. Hahn is gratefully acknowledged. This research project was supported in part by a grant of the Deutsche Forschungsgemeinschaft (SFB367/C1).

References

1. Barton RW, Prendergast J, Kennedy CA (1990) Binding of the T cell activation monoclonal antibody Ta1 to dipeptidyl peptidase IV. J Leu Biol 48:291–296
2. Cohen J (1993) T cell shift: key to AIDS therapy? Science 262:175–176
3. Cordell JL, Fallini B, Erber WN, Ghosh AK, Abdulaziz Z, MacDonald S, Pulford KAF, Stein H, Mason DY (1984) Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase antibodies (APAAP complexes). J Histochem Cytochem 32:219–229
4. Dang NH, Torimoto Y, Deusch K, Schlossman SF, Morimoto C (1990) Comitogenic effect of solid-phase immobilized anti-1F7 on human CD4 T cell activation via CD3 and CD2 pathway. J Immunol 144:4092–4100
5. Duke-Cohan JS, Morimoto C, Rocker JA, Schlossman SF (1996) Serum high molecular weight dipeptidyl peptidase IV (CD26) is similar to a novel antigen DPPT-L released from activated T cells. J Immunol 156:1714–1721
6. Fox DA, Hussey RE, Fitzgerald KA, Acuto O, Poole C, Palley L, Daley JF, Schlossman SF, Reinherz EL (1984) Ta1, a novel 105 kD human T cell activation antigen defined by a monoclonal antibody. J Immunol 133:1250–1256
7. Gorrell MD, Levy M, Abbott CA, Washington EA, McCaughan GW, Morrow AW (1996) CD26: ADA binding, immunoblotting and species cross reactivity examined using recombinant proteins. Tissue Antigens 48:451
8. Hegen M, Dong R-P, Morimoto C, Schlossman SF (1996) Screening the non-lineage (N-L) section monoclonal antibodies: mapping of epitopes of the N-L anti-CD26 monoclonal antibodies and adenosine deaminase binding region on CD26 transfectants. Tissue Antigens 48:451
9. Heinzel FP, Sadick MD, Holaday BJ, Coffman RL, Locksley RM (1989) Reciprocal expression of interferon-γ or interleukin-4 during the resolution or progression of murine leishmaniasis: evidence for expansion of distinct helper T cell subsets. J Exp Med 169:59–72
10. Jacotot E, Callebaut C, Blanco J, Krust B, Neubert K, Barth A, Hovanessian AG (1996) Dipeptidyl-peptidase IV-β, a novel form of cell-surface-expressed protein with dipeptidyl-peptidase IV activity. Eur J Biochem 239:248–258
11. Kähne T, Bühling F, Lendeckel U, Reinhold D, Taeger M, Wrenger S, Faust J, Neubert K, Ansorge S (1996) The effect of anti-CD26 antibodies on DNA synthesis and cytokine production (IL-2, IL-10 and IFN-γ) depends on enzymatic activity of CD26/DPIV. Tissue Antigens 48:451
12. Kähne T, Kroning H, Thiel U, Ulmer AJ, Flad HD, Ansorge S (1996) Alterations in structure and cellular localization of molecular forms of DPIV/CD26 during T cell activation. Cell Immunol 170:63–70
13. Kreisel W, Hanski C, Tran-Thi T-A, Katz N, Decker K, Reutter W, Gerok W (1988) Remodeling of a rat hepatocyte plasma membrane glycoprotein. De- and reglycosylation of dipeptidyl peptidase IV. J Biol Chem 263:11736–11742
14. Mattern T, Scholz W, Feller AC, Flad H-D, Ulmer AJ. Expression of CD26 (dipeptidyl peptidase IV) on resting and activated human T-lymphocytes. Scand J Immunol 33:737–748
15. Modlin RL, Melancon-Kaplan J, Young SMM, Pirmez C, Kino H, Convit J, Rea TH, Bloom B (1988) Learning from lesions: patterns of tissue inflammation in leprosy. Proc Natl Acad Sci USA 85:1213–1217
16. Mosmann TR, Coffman RL (1989) Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 7:145–173
17. Mosmann TR, Sad S (1996) The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today 17:138–146
18. Reinhold D, Bank U, Bühling F, Kähne T, Kunt D, Faust J, Neubert K, Ansorge S (1994) Inhibitors of dipeptidyl IV (DP IV, CD26) specifically suppress proliferation and modulate cytokine production of strongly CD26 expressing U937 cells. Immunobiology 192:121–136
19. Ridley DS, Jopling WH (1966) Classification of leprosy according to immunity. A five group system. Int J Leprosy 34:255–273
20. Romagnani S (1991) Human Th1 and Th2 subsets: doubt no more. Immunology Today 12:256–257

21. Scheel D, Toellner K-M, Reiling N, Ernst M, Ulmer AJ, Flad H-D, Gerdes J (1993) Enrichment of Th-1 cells from human peripheral blood by three colour immunofluorescence staining for CD4, CD45RO and CD26. Tissue Antigens 42: 250
22. Scheel D, Richter E, Toellner K-M, Reiling N, Key G, Wacker H-H, Ulmer AJ, Flad H-D, Gerdes J (1995) Correlation of CD26 expression with Th1-like reactions in granulomatous diseases. In: Schlossman SF, Boumsell L, Gilks WR, Harlan JM, Kishimoto T, Morimoto C, Ritz J, Shaw S, Siverstein R, Springer T, Tedder TF, Tod RF (eds) Leucocyte typing V: white cell differentiation antigens. Oxford University Press, Oxford, pp 1111–1114
23. Scheel-Toellner D, Richter E, Toellner K-M, Reiling N, Key G, Ballestren W Graf von, Ulmer AJ, Wacker H-H, Flad H-D, Gerdes J (1995) CD26 expression in leprosy and other granulomatous diseases correlates with the production of interferon-gamma. Lab Invest 73:685–690
24. Seder RA, Paul WE (1994) Acquisition of lymphokine-producing phenotype by CD4+ T cells. Annu Rev Immunol 12:635–673
25. Stein H, Schwarting R, Niedobitek G (1989) Cluster report: CD26. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, von dem Borne AEGKr (eds) Leucocyte typing IV: white cell differentiation antigens. Oxford University Press, Oxford, pp 412–418
26. Tanaka T, Camerini D, Seed B, Torimoto Y, Dang NH, Kameoka J, Dahlberg HN, Schlossman SF, Morimoto C (1992) Cloning and functional expression of the T cell activation antigen CD26. J Immunol 149:481–486
27. Torimoto Y, Dang NH, Tanaka T, Prado C, Schlossman SF, Morimoto C (1992) Biochemical characterization of CD26 (dipeptidyl peptidase IV): functional comparison of distinct epitopes recognized by various anti-CD26 monoclonal antibodies. Mol Immunol 29:183–192
28. Willheim M, Ebner C, Baier K, Kern W, Schrattbauer K, Thien R, Kraft D, Breiteneder H, Reinisch W, Scheiner O (1997) Cell surface characterization of T lymphocytes and allergen-specific T cell clones: Correlation of CD26 expression with Th1 subsets. J Allergy Clin Immunol 100:348–355
29. Yamamura M, Uyemura K, Deans RJ, Weinberg K, Rea TH, Bloom BR, Modlin RJ (1991) Defining protective responses to pathogens: cytokine profiles in leprosy lesions. Science 254:277–279