Analysis of Differentiation Marker Proteins in Helper T Cell Subtypes, Th1 and Th2

Jun-ichi Kashiwakura, Akiko Suzuki, Kaori Saitoh, Shozo Yamashita, and Satoshi Toyoshima[1,*]

Department of Biochemistry, Hoshi University, 2–4–41 Ebara, Shinagawa-ku, Tokyo 142–8501, Japan

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Human helper T cells are divided into T helper (Th) 1 and Th2 cells, which are known to have important roles in cell-mediated and humoral immunity, respectively. In the present study, we attempted to identify cell markers that can distinguish Th1 and Th2 cells using large-scale two-dimensional electrophoresis. Several proteins were found to be specifically and reproducibly expressed in each helper T cell subtype. Three proteins, which have molecular weights of 35000 (spot 1), 40000 (spot 2) and 49000 (spot 3) with pIs of 7.0, 7.0 and 6.3, respectively, were found only in Th1 cells, and the other three proteins, which have molecular weights of 32500 (spot 4), 38000 (spot 5) and 44000 (spot 6) with pIs of 6.5, 6.0 and 5.8, respectively, only in Th2 cells. Spots 1, 3 and 4 were found in both the membrane and the cytosol. Spots 2 and 5 were membrane proteins, while spot 6 was a cytosol protein. The molecular weight of spot 4 resembled that of ST2, which was reported to be a specific cell surface marker of Th2 cells. However, anti-ST2 antibody did not react with spot 4 at all. These results suggest that the proteins found in the present study are novel cell markers of Th1 and Th2 cells.

Key words —— helper T cells, Th1, two-dimensional electrophoresis

INTRODUCTION

Differentiation of human helper T cells into Th1 and Th2 cells is vital for the development of cell-mediated and humoral immunity, respectively. Th1 cells, which are characterized by the secretion of cytokines such as interferon (IFN)-γ are associated with cellular immunity including macrophage cytotoxicity and delayed type hypersensitivity.[1,2] Th2 cells, which are characterized by the secretion of cytokines such as interleukin (IL)-4 and IL-5, are associated with humoral immunity.[3] It has been suggested that cytokines and their receptors, transcription factors, major histocompatibility complex (MHC) determinants, signal transducers, antigen-peptides and co-stimulatory signals are important for Th1 and Th2 cell differentiation.[4–15] For example, Txk, a non-receptor tyrosine kinase, which is reported to regulate IFN-γ production, is expressed in Th1 cells but not in Th2 cells.[9]

In recent studies, several membrane proteins have been shown to be cell surface markers of Th1 and Th2 cells.[10,16–19] CD30 is selectively expressed on cloned Th2 cells,[19,20] and CCR3, which belongs to the chemokine receptor family, is also selectively expressed on Th2 cells.[21,22] On the other hand, the IL-12 receptor β2 subunit has been detected only on the surface of Th1 cells stimulated with IL-12 and IFN-γ.[23–27] CCR5 is expressed on Th1 cells but not on Th2 cells.[17,18,28] Moreover, lymphocyte activation gene-3 (LAG-3) is expressed on Th1/0 cells that are affected by IFN-γ.[10] However, useful marker proteins to distinguish between Th1 and Th2 cells have not been established yet.

Therefore, in the present study, to identify useful marker proteins that can distinguish Th1 and Th2 cells, we compared cellular proteins of Th1 cells with those of Th2 cells by using a large-scale two-dimensional gel electrophoresis system. Several proteins were found to be specifically or selectively expressed in Th1 or Th2 cells, and some of these were membrane proteins. One of Th2-specific proteins had characteristics similar to those of ST2 protein, which is specifically expressed in Th2 cells,[29] but this was not identical with ST2 protein

[1]Present address: Pharmaceuticals and Medical Devices Evaluation Center, National Institute of Health Sciences, Mori-building 33, Toranomon 3–8–21, Minato-ku, Tokyo 105–8409, Japan.
*To whom correspondence should be addressed: Pharmaceuticals and Medical Devices Evaluation Center, National Institute of Health Sciences, Mori-building 33, Toranomon 3–8–21, Minato-ku, Tokyo 105–8409, Japan. Tel.: +81-3-5403-1411; Fax: +81-3-5403-1417; E-mail: toyosima@nihs.go.jp

MATERIALS AND METHODS

Cells —— Several human T cell clones to various specific antigens (Ag-specific T cell clones) were used in this study. These clones were specific to house dust mite (HDM), Japanese cedar (Cryptomeria japonica) pollen 1 (Cry j 1), and purified protein derivatives of tuberculosis (PPD). The clones were kindly provided by Dr. T. Sakane (Departments of Immunology and Medicine, St. Marianna University School of Medicine).

Large-Scale Two-Dimensional Gel Electrophoresis —— Th1 and Th2 cells were washed and lysed in sample buffer 1 containing 7.92 M Urea, 3.2% NP-40, 1.76% Ampholyte, 120 mM DTT, 10 mM Tris–HCl (pH 7.8) and 0.06% SDS. A lysate (8.3×10^5 cells) was separated on Immobiline DryStrip (pH 3–10: Amersham Pharmacia Biotech, Piscataway, NJ, U.S.A.) in the first dimension. Next, the samples on the Immobiline were separated by 12.5% SDS-PAGE in the second dimension. Then gels were stained by 2-D silver stain II "DAIICHI" (Daiichi Pure Chemicals Co., Ltd., Japan) and analyzed by PDQUEST (PDI, Huntington Station, NY, U.S.A.).

Isolation of Peripheral Blood T Cells —— Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood of healthy volunteers on Ficoll-Hypaque density gradients. After washing, the cells were resuspended at 5×10^7 cells/ml in RPMI 1640 medium (ICN Biomedicals Inc., Aurora, OH, U.S.A.). Then, T cells were purified from PBMCs by the nylon wool column method.[30]

Separation of Cell Membrane and Cytosol —— T cells were suspended in TE buffer containing 0.1 M Tris–HCl (pH7.5) and 5 mM EDTA and sonicated by the supersonic wave technique. The mixture was centrifuged ($12000 \times g$, 20 min). The supernatant was concentrated and stocked as cytosol, and the pellet was as cell membranes. The cytosol and membrane were dissolved in sample buffer 1 at 10^7 cells/80 μl and Urea TX buffer containing 9 M Urea, 2% TritonX-100, 1% DTT and 2% Ampholyte at 10^7 cells/100 μl, respectively. These samples were analyzed by the two-dimensional electrophoresis system as described above.

Western Blotting —— Anti-ST2 antibody (Ab) was obtained from rabbits immunized with a peptide (GQARIQEEEGRNESSSNDMDC) as described by Kikuchi *et al*.[31] Samples were dissolved in Urea TX buffer, and analyzed by two-dimensional gel electrophoresis. After electrophoresis, samples were transferred to nitrocellulose membranes (Hybond ECL, Amersham Pharmacia Biotech, Buckinghamshire, U.K.). For protein detection, membranes were blocked with Block Ace (Dainippon Pharmaceutical Co., Ltd., Japan) and treated with the anti-ST2 Ab as the primary reagent and with anti-rabbit Ab linked to HRP (Dainippon Pharmaceutical Co., Ltd.) as the secondary reagent. The binding of HRP-linked Ab was detected by ECL (Amersham Pharmacia Biotech).

RESULTS AND DISCUSSION

To investigate useful marker proteins to distinguish Th1 cells from Th2 cells, cellular proteins of Th1 and Th2 cells were analyzed by a large-scale two-dimensional electrophoresis system. First, whole proteins of Th1 cells were compared with those of Th2 cells. Figure 1 shows a superposition of the spots from both gels. A total of 1730 distinct protein spots were obtained with Th1 cells and Th2 cells. Of these spots, 627 were present in both cells (green spots), 378 were specific to Th1 cells (blue spots) and 725 were specific to Th2 cells (red spots).

After performing the two-dimensional electrophoresis several times, only six spots that were specifically or selectively expressed in Th1 or Th2 cells were found to be reproducible. Three proteins with molecular weights of 35000 (spot 1), 40000 (spot 2) and 49000 (spot 3) with pIs of 7.0, 7.0 and 6.3, respectively, were found to be specifically or selectively expressed in Th1 cells (Fig. 2A), and three other proteins with molecular weights of 32500 (spot 4), 38000 (spot 5) and 44000 (spot 6) with pIs 6.5, 6.0 and 5.8, respectively, were found to be specifically or selectively expressed in Th2 cells (Fig. 2B). Spots 1 and 4 were specifically expressed in Th1 cells and Th2 cells, respectively, spots 2 and 3 were more strongly expressed in Th1 cells than in Th2 cells, and spots 5 and 6 were more strongly expressed in Th2 cells than in Th1 cells (Table 1).

Most of the known marker proteins of helper T cell subtypes have been reported to be membrane proteins,[21,22,28,32] while Txk, IRF-1 and ST2 have been shown to be cytosol proteins.[9,33,34] Furthermore, Txk is also located in the nucleus.[9] Therefore, the cellular location of specifically or selectively expressed proteins in Th1 or Th2 cells was studied. Spot 5 was detected only in the membrane fraction from purified T cells (Fig. 3A), while spots 2 and 6 were detected only in the cytosol fraction (Fig. 3B).

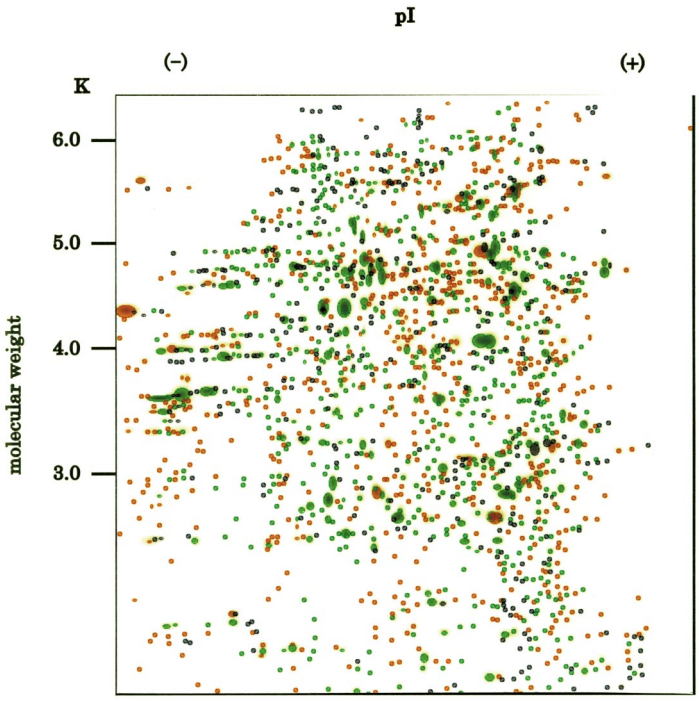

Fig. 1. Comparison of Proteins Expressed in Ag-Specific Th1 and Th2 Cells

Ag-specific Th1 and Th2 cells (8.3×10^5 cells) were lysed in sample buffer 1 (20 μl) and analyzed by the two-dimensional gel electrophoresis system. Then, gels were stained with silver staining kits. These gels were analyzed by PDQUEST. Green spots, spots expressed in both Th1 and Th2 cells; Blue spots, spots expressed predominantly in Th1 cells; Red spots, spots expressed predominantly in Th2 cells.

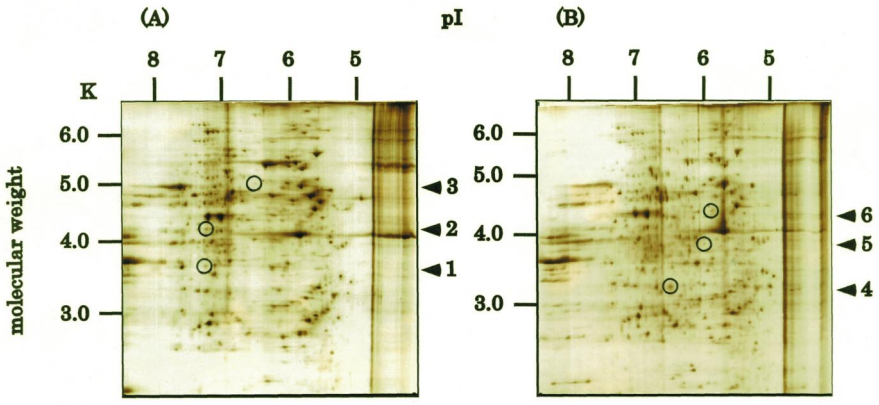

Fig. 2. Analysis of Proteins Expressed Only or Predominantly in Th1 or Th2 Cells

Total proteins expressed in Th1 or Th2 cells were separated by the two-dimensional gel electrophoresis system, and were detected by silver staining. Arrowheads show proteins expressed only or predominantly in Th1 or Th2 cells. Gel images of Th1 cells and Th2 cells are shown in A and B, respectively.

Table 1. Characteristics of Th1 and Th2 Specific Proteins

Spot	MW	pI	Expression in	Location	
			Th cells	Membrane	Cytosol
1	35000	7.0	Th1	+	+
2	40000	7.0	Th1 ≫ Th2	−	+
3	49000	6.3	Th1 ≫ Th2	+	+
4	32500	6.5	Th2	+	+
5	38000	6.0	Th2 ≫ Th1	+	−
6	44000	5.8	Th2 ≫ Th1	−	+

Molecular weight (MW) and pI of spot 1–6 based on the results of two-dimensional gel electrophoresis.

On the other hand, spots 1, 3 and 4 were detected in both the membrane and cytosol fractions (Fig. 3, Table 1). These results suggest that spot 5 is a membrane protein and spots 2 and 6 are cytosol proteins.

In the present study, six proteins have been shown to be specifically or selectively expressed in Th1 or Th2 cells and some of their characteristics are also shown. Next, to investigate whether some of these six proteins are identical with known marker proteins of Th1 or Th2 cells, the characteristics of

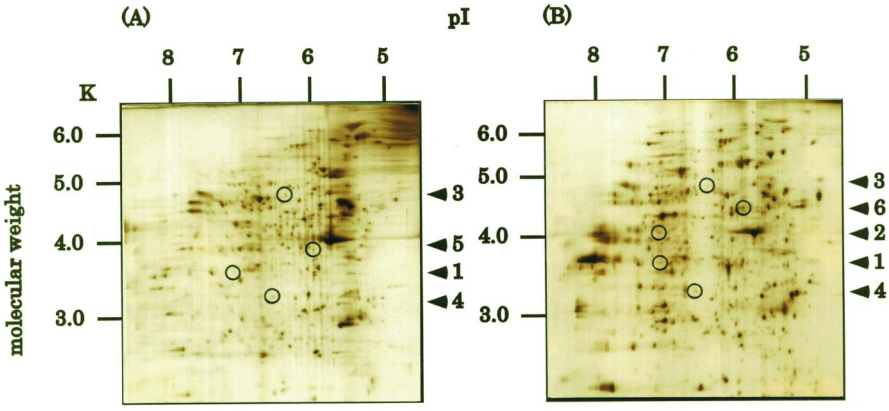

Fig. 3. Location of Proteins Specifically or Selectively Expressed in Th1 or Th2 Cells

Ag-specific Th1 and Th2 cells were washed, and resuspended with TE buffer. Then, cells were sonicated and separated into soluble and insoluble fractions. Concentrated soluble fractions and insoluble fractions were resuspended in sample buffer 1 or Urea TX buffer, respectively, and analyzed by the two-dimensional gel electrophoresis system. Arrowheads show detected spots. Gel images of cell membrane and cytosol are shown in A and B, respectively.

Table 2. Details of Known Marker Proteins in Th1 and Th2 Cells

Th1 specific proteins

Name	Location	Speculated values		Reported values		References
		MW	pI	MW	pI	
LAG-3	Membrane	57494.61	8.07			35
CCR5	Membrane	40523.43	9.11	38 kD		36, 37
Txk	Cytosol	61238.37	7.72	64 kD		9, 38
IL-12R β2	Membrane			130 kD		39
IL-18R β	Membrane	68308.50	8.33			40
IRF-1	Cytosol	36501.64	5.09	37.3 kD		41
CXCR3	Membrane	4934.36	6.74			42

Th2 specific proteins

Name	Location	Speculated values		Reported values		References
		MW	pI	MW	pI	
GATA-3	Membrane	48044.27	9.61			43
CCR3	Membrane	41043.16	8.15			44
CD30	Membrane	63746.27	5.33	88–120 kD		19, 45
ST2L	Membrane	63414.99	8.19	69–80 kD		34, 46
ST2	Cytosol	37050.53	7.85	60 kD (oligomer)		46, 47
CCR4	Membrane	41402.19	7.35	37.3 kD		48

Molecular weight (MW) and pI of proteins reported to be cell surface markers that distinguish between Th1 and Th2 cells. MWs and pIs were predicted by GENETYX.

proteins of spots 1–6 (Table 1) were compared with those of known marker proteins (Table 2). The molecular weights of spots 1 and 2 were similar to those of IRF-1 and CCR5, respectively, and the molecular weights of spots 4 and 5 were similar to the molecular weight of ST2. On the other hand, pI values of these spots were different from those of IRF-1, CCR5 and ST2, respectively. Moreover, the localization of spot 5 is different from that of ST2, a cytosol protein (Table 1). These results suggest that the six proteins identified in this study do not correspond with known marker proteins of Th1 or Th2 cells. However, since the molecular weights of two spots (spots 4 and 5) were similar to the molecular weight of ST2, and since ST2 is known to be related to the production of Th2-type cytokines,[29,49] we further examined spots 4 and 5 by western blot analysis to see if either is identical to ST2 (Fig. 4). For this

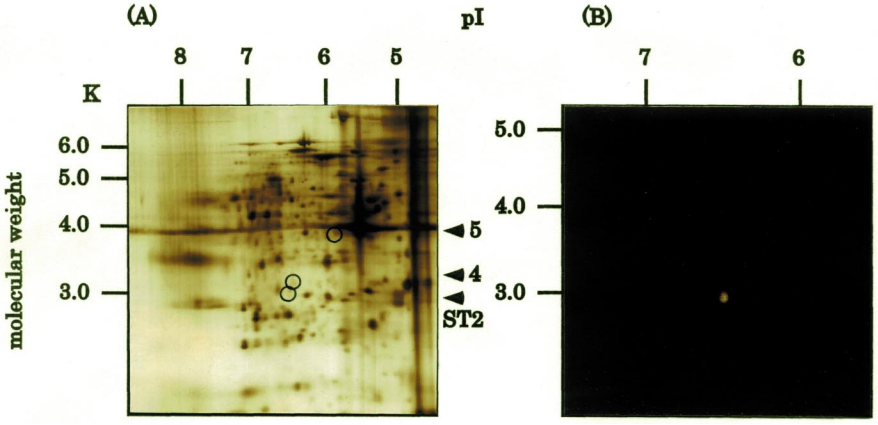

Fig. 4. Two-Dimensional Gel Electrophoresis of T cell Proteins and Western Blotting of ST2

Purified T cells were resuspended in Urea TX buffer and analyzed by the two-dimensional gel electrophoresis. (A) Gel image of T cell proteins by two-dimensional gel electrophoresis. Arrowheads show spots 4 and 5 and ST2 protein. In this study, the spot detected by anti-human ST2L Ab had the molecular weight of 30000 and pI of 6.5. (B) T cell proteins were separated by the two-dimensional gel electrophoresis. Then, proteins on the gel were transferred to nitro cellulose membranes and analyzed by western blotting using anti-human ST2 Ab. The white spot in Fig. 4B is ST2.

analysis, we used an antibody specific for ST2 and ST2L. ST2 is a soluble form of ST2L, which is a member of IL-1 receptor family, and has been suggested to be a useful marker of Th2 cells.[29,34,50] ST2 was detected (Fig. 4B, MW = 30000, pI = 6.5), but the position of this spot didn't correspond with that of spot 4 or 5 in Fig. 4A. This result suggests that spots 4 and 5 may be novel markers that can be used to distinguish between Th1 and Th2 cells. The six proteins identified in this study do not seem to be identical with known marker proteins such as CCR5 and ST2 (Table 1 and 2). Recently, Nagata *et al*. reported that a receptor protein, CRTH2 is selectively expressed in activated Th2 cells and suggested that this receptor has a pivotal role in Th2-type immune reactions.[51] CRTH2 is a novel member of the G protein-coupled leukocyte chemoattractant receptor family. It will be interesting to learn whether CRTH2 is one of the six proteins identified in this study. However, the molecular weights of these proteins were lower than the molecular weight of CRTH2, which is 55000 to 70000.

Further studies of the six proteins identified in this study are now being conducted to distinguish Th1 cells from Th2 cells.

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