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Early Events of TCR Signaling Are Distinct in Human Th1 and Th2 Cells

Sigrid Hannier,1 Christina Bitegye, and Stéphane Demotz2

To study the requirements for activation of human Th1 and Th2 cells, soluble peptide/DR1 complexes were prepared from naturally expressed DR1 protein. When immobilized, this material induced T cell activation, as revealed by CD25 up-regulation. Unexpectedly, Th2 cells required a higher density of peptide/DR1 complexes than Th1 cells to initiate CD25 up-regulation. Similar findings were obtained with immobilized or soluble and cross-linked anti-CD3 mAb. In contrast, peptide/DR1 complexes displayed on the surface of nonprofessional APC similarly induced CD25 up-regulation in Th1 and Th2 cells. Signaling events distinguishing human Th1 and Th2 cells following TCR engagement by anti-CD3 mAb were then studied. It was observed that upon TCR triggering, the overall tyrosine phosphorylation profiles were fainter in Th2 than in Th1 clones. Similar results were obtained with Th1- and Th2-polarized polyclonal lines. Varying the dose of anti-CD3 mAb, the kinetics of activation, and coengagement of CD3 and CD28 failed to increase tyrosine phosphorylation in Th2 cells to levels reached in Th1 cells. In contrast, treatment with the tyrosine phosphatase inhibitor phenylarsine oxide resulted in similar tyrosine phosphorylation levels in Th2 and Th1 cells. These findings indicated that Th2 cells had an intrinsically lower TCR-induced tyrosine phosphorylation capacity than Th1 cells, which might be controlled by Th1- and Th2-specific phosphatase profiles. Finally, a weaker association was found between ZAP-70 and CD3ζ in Th2 than in Th1 cells after TCR engagement. Taken together, these results constituted evidence that early events in the TCR signaling cascades are distinct in human Th1 and Th2 cells. The Journal of Immunology, 2002, 169: 1904–1911.

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CD3 mAb. A biochemical analysis of TCR signaling events induced by anti-CD3 Ab revealed fainter tyrosine phosphorylation profiles and a weaker CD3ζ/ZAP-70 association in human Th2 than in Th1 cells. Distinct proximal events of the TCR signaling cascades between Th1 and Th2 cells might be due to distinct profiles of phosphatases found in Th1 and Th2 cells.

Materials and Methods

Cell clones and lines

PBMC were obtained by Ficoll-Paque (AmershamsPharmacia Biotech, Orsay, France) density gradient centrifugation of heparinized blood from healthy donors. Measles virus fusion protein derived peptide 254–268 (F254)-specific, HLA-DRB1*0101-restricted Th1 and Th2 clones were isolated from PBMC (1.5 × 10^6 cells/200 µl) cultured for 1 wk in the presence of 100 ng/ml of F254 peptide. The cultures were performed under Th1- and Th2-polarizing conditions as previously described (18). Briefly, for differentiation of Th1 cells, cultures were supplemented with 2 ng/ml IL-12 (BD Biosciences, San Jose, CA) and 125 ng/ml anti-IL-4 mAb (clone 8D4-8; BD Biosciences). For differentiation of Th2 cells, cultures were supplemented with 7 ng/ml IL-4 (R&D Systems, Minneapolis, MN) and 2 µg/ml anti-IL-12 mAb (clone C8.6; BD Biosciences). Isolation and maintenance of the Th clones were previously reported in detail (19). The three Th1 (F254.7.2, SYMV13, and Th1 no. 4) and three Th2 (F6R.6.6, SDMV3.5, and Th2 no. 1) clones used in this study were isolated from independent cultures, and from two donors.

T cell lines were established by culture of PBMC at 10^6 cells/ml for 2 wk with 10 ng/ml toxic shock syndrome toxin superantigen (Sigma, Saint Quentin Fallavier, France) in the presence of cytokines and mAb for differentiation into Th1 and Th2 cells, as described for the T cell clones.

Th1 and Th2 polarization was determined by ELISA. Cells producing IFN-γ, but not IL-5, were considered Th1, and cells producing IL-5, but not IFN-γ, were considered Th2. Since differentiation of Th2 cells was conducted in medium containing IL-4, IL-5, rather than IL-4, was determined to establish Th phenotypes.

T cells were cultured in RPMI medium (Life Technologies, Cergy Pontoise, France) supplemented with glutamine, sodium pyruvate, nonessential amino acids, kanamycin, 5% decomplemented AB human serum, and 100 U/ml IL-2 (Tebu, Le Perray en Yvelines, France). Th1 and Th2 cells were simultaneously restimulated for expansion, and were used at least 15 days after restimulation.

The HLA-DR1-transfected HeLa cell line was cultured in RPMI medium supplemented with glutamine, sodium pyruvate, nonessential amino acids, kanamycin, 10% decomplemented FCS, and 1 ng/ml genetin (Life Technologies) (20).

Antibodies and chemicals

For T cell stimulation, anti-human CD3 mAb TR66 (mouse IgG1) was a gift from Dr. Lanzavecchia (Biomedical Research Institute, Bellinzona, Switzerland). Anti-human CD28 mAb (clone CD28.2, mouse IgG1) and anti-p38 mAb were purchased from Euromedex (Souffelweyersheim, France); anti-p44/p42 MAPK mAb was obtained from Serotec (Oxford, UK). Goat anti-mouse IgG Ab was obtained from Tebu. Anti-human CD28 mAb (clone CD28.2, mouse IgG1) and isotype-matched control were incubated with 3 × 10^8 cells/ml for 1 h at 4°C in PBS; 0.1% sodium azide, and 1% FCS, then washed twice in PBS. Mean fluorescence was measured by flow cytometry on an EPICS XL cytometer (Coulter, Miami, FL).

Cell stimulation and analysis

Cells were washed twice, resuspended at 12 × 10^6 cells/ml in prewarmed RPMI and incubated in a water bath at 37°C for 15 min. Stimulation was then performed on 6 × 10^5 cells/ml by addition of soluble TR66 mAb, in the presence of the absence of anti-CD28 mAb, followed by addition of goat anti-mouse IgG Ab and incubation at 37°C for the indicated periods of time. Stimulation was stopped by centrifugation, followed by solubilization of the cell pellets for 45 min on ice in 100 µl lysis buffer (1% Igepal (Sigma) in 20 mM Tris, pH 7.5) and 140 mM NaCl, supplemented with 1 mM EDTA, 1 mM sodium orthovanadate, and a mixture of protease inhibitors (Mini Complete; Roche Diagnostics) used according to the manufacturer’s instructions. Each condition of stimulation was simultaneously performed on Th1 and Th2 cells. After removal of nuclei and cellular debris by centrifugation, the protein concentration was determined (bicinchoninic acid; Pierce, Interchim, Asnières, France), and constant amounts of protein sample were diluted in Laemml sample buffer (Bio-Rad, Marne la Coquette, France) with 5% 2-ME and boiled for 3 min before SDS-PAGE analysis.

Lipid raft fractionation

Cell lysates (15 × 10^6 cells/ml) were prepared as described above in 1 ml lysis buffer, except that 1% Triton X-100 was used as a detergent. The samples were centrifuged through a sucrose step gradient as described by Xavier et al. (22). Briefly, following 30 min incubation on ice, the lysates were gently mixed with 1 ml 85% sucrose in lysis buffer. The samples were placed in the bottom of a tube, overlaid by 6 ml 35% sucrose, followed by 4 ml 5% sucrose in lysis buffer, and centrifuged in a SW40 Beckman rotor (Fullerton, CA) for 16 h at 180,000 × g at 4°C without stopping. The gradients were separated into 11 1.2-ml fractions by collection from the bottom of the tube. Each fraction was precipitated by addition of 0.2 ml 100% TCA in water, and the pellet was washed twice with acetone. The pellets were resuspended in Laemmli sample buffer plus 5% 2-ME and boiled for 3 min before SDS-PAGE analysis. One-fifth of each fraction was loaded on 12% SDS-PAGE gels.

Immunoprecipitations

Cell lysates (6 × 10^6 cells/ml) were prepared for analysis as described above in 1 ml lysis buffer, except that 1% Triton X-100 was used as a detergent. The samples were centrifuged through a sucrose step gradient as described by Xavier et al. (22). Briefly, following 30 min incubation on ice, the lysates were gently mixed with 1 ml 85% sucrose in lysis buffer. The samples were placed in the bottom of a tube, overlaid by 6 ml 35% sucrose, followed by 4 ml 5% sucrose in lysis buffer, and centrifuged in a SW40 Beckman rotor (Fullerton, CA) for 16 h at 180,000 × g at 4°C without stopping. The gradients were separated into 11 1.2-ml fractions collection from the bottom of the tube. Each fraction was precipitated by addition of 0.2 ml 100% TCA in water, and the pellet was washed twice with acetone. The pellets were resuspended in Laemmli sample buffer plus 5% 2-ME and boiled for 3 min before SDS-PAGE analysis. One-fifth of each fraction was loaded on 12% SDS-PAGE gels.

Immunoblotting

Equal amounts of protein, as determined by Bradford assay (Bio-Rad), were run on 12% SDS-PAGE gels and then electrophoresed onto nitrocellulose (Bio-Rad). The membranes were washed in 20 mM Tris (pH 7.4) and 150 mM NaCl (TBS) containing 3% gelatin (Bio-Rad). Immunoblotting was performed using the indicated Ab, followed by HRP-labeled goat anti-mouse Ab or goat anti-rabbit IgG Ab in TBS, 0.1% Tween 20, phosphatase 1.
CD25 up-regulation is more sensitive to immobilized TCR ligands in Th1 than in Th2 cells

Isolated F254 peptide/DR1 complexes offered the opportunity to compare activation of Th1 and Th2 cells following engagement of the TCR by their physiological ligands in the absence of the contribution of accessory and adhesion molecules. As assessed by the percentage of CD25 up-regulation relative to CD25 expression by nonstimulated cells, activation in three F254-specific, DR1-restricted Th1 clones was elicited by at least 10 times less immobilized F254 peptide/DR1 complexes than in three Th2 clones exhibiting the same Ag and restriction specificity (Fig. 2A). To facilitate comparison between clones, since the magnitude of CD25 up-regulation was higher in Th2 than in Th1 cells, results were expressed as the percentage of CD25 up-regulation (23). Biochemical analyses of signaling events triggered by immobilized F254 peptide/DR1 complexes were foreseen as technically difficult. For that reason we evaluated whether the use of anti-CD3 mAb, a more suitable reagent for the purpose of signal transduction studies, resulted in similar activation profiles distinguishing Th1 and Th2 cells. Similar to the previous situation, 100-fold lower amounts of immobilized anti-CD3 mAb induced CD25 up-regulation by Th1 than by Th2 cells (Fig. 2B). Likewise, stimulation with soluble anti-CD3 mAb cross-linked with anti-mouse IgG Ab resulted in a 10-fold more sensitive dose-response in Th1 than in Th2 cells (Fig. 2B). However, it could not be formally excluded that a fraction of soluble Abs might be adsorbed on the plastic wells and contributed to T cell activation. Nevertheless, in both conditions CD25 up-regulation was triggered by less anti-CD3 mAb in Th1 than in Th2 cells. These results indicated that TCR engagement resulted in distinct signaling events in Th1 and Th2 cells regardless of the form of the anti-CD3 mAb, immobilized or soluble and cross-linked. Similar results were obtained with the other Th1 and Th2 clones used in this study (data not shown).

F254 peptide/DR1 complexes displayed on HeLa cells elicit similar CD25 up-regulation in Th1 and Th2 cells

Remarkably, when Th1 and Th2 cells were triggered with F254 peptide-pulsed DR1-transfected HeLa cells, the two Th subsets did not exhibit marked differences in their sensitivities. The most sensitive clones were the Th2 FR6.6 and Th1 6396 F254.7.2 clones. The four other Th1 and Th2 clones showed similar Ag dose responses (Fig. 3). Stimulation by F254 peptide-pulsed HeLa cells...
The distinct stimulation thresholds of Th1 and Th2 cells obtained with immobilized TCR ligands prompted us to evaluate whether TCR signaling pathways differed in these two types of cells. Profiles of tyrosine phosphorylation exhibited by Th1 and Th2 cells were analyzed by Western blotting as a first step aimed at assessing multiple signaling events following TCR engagement. Strikingly, stimulation with soluble and cross-linked anti-CD3 mAb led to overall fainter phosphotyrosine profiles in Th2 than in Th1 cells. In parallel, p38 mitogen-activated protein kinase was Western blotted to determine that equal amounts of protein were loaded in each lane (Fig. 4A). Similarly, faint phosphotyrosine profiles were observed before and after TCR engagement in three other Th2 clones compared with three other Th1 clones (data not shown). Suboptimal TCR engagement also resulted in fainter phosphotyrosine profiles in Th2 than in Th1 polyclonal lines, indicating that following comparable levels of TCR ligation, a more sustained cascade of signaling events was generated in Th1 than in Th2 cells (Fig. 4B). Tyrosine phosphorylation profiles were also fainter in Th2 than in Th1 cells regardless of the time following TCR engagement. Maximal intensity in both Th1 and Th2 cells was observed at 150 s after TCR ligation, followed by decay of the phosphotyrosine signal (Fig. 4C). This finding was observed for three Th1 and three Th2 clones (data not shown). Together these data established that anti-CD3 mAb-mediated TCR engagement resulted in a lower phosphotyrosine content in Th2 than in Th1 cells. These differences could not be explained by distinct optimal intensities of stimulation or by different response kinetics. Rather, they indicated that Th2 cells were inherently less capable than Th1 cells of anti-CD3 mAb TCR-induced tyrosine phosphorylation.

**CD3 and CD28 coengagement fails to increase tyrosine phosphorylation in Th2 cells to levels reached in Th1 cells**

It is well documented that signaling pathways emanating from the TCR and CD3 complex alone are not sufficient for T cell activation. Simultaneous engagement of costimulatory molecules, in particular CD28, is required to achieve full activation of T cells. Therefore, it was evaluated whether simultaneous engagement of CD3 and CD28 increased tyrosine phosphorylation in Th2 cells to levels matching those attained in Th1 cells. First, immunofluorescence staining established that expression levels of CD28 were similar in Th1 and Th2 clones (data not shown). Together these data established that anti-CD3 mAb-mediated TCR engagement resulted in a lower phosphotyrosine content in Th2 than in Th1 cells. These differences could not be explained by distinct optimal intensities of stimulation or by different response kinetics. Rather, they indicated that Th2 cells were inherently less capable than Th1 cells of anti-CD3 mAb TCR-induced tyrosine phosphorylation.

**The tyrosine phosphatase inhibitor phenylarsine oxide reverts faint phosphotyrosine profiles of Th2 cells**

An intrinsic low kinase activity in Th2 compared with Th1 cells might explain the faint intensity of the phosphotyrosine profiles after TCR engagement in Th2 cells. Alternatively, the phosphatase activity might be higher in Th2 than in Th1 cells. As a result, tyrosine phosphorylation would be more inhibited in Th2 than in Th1 cells. To discriminate between these two hypotheses, Th1 and Th2 cells...
were stimulated with anti-CD3 mAb in the presence or the absence of phosphatase inhibitors. Remarkably, the tyrosine phosphorylation profiles in Th2 cells treated with the tyrosine phosphatase inhibitor phenylarsine oxide were as intense as those in Th1 cells similarly treated, while the serine and threonine phosphatase inhibitor okadaic acid failed to restore intense tyrosine phosphorylation profiles in Th2 cells after TCR engagement (Fig. 6A). Analysis of three Th1 and three Th2 clones gave the same results (data not shown). This circumstantial evidence indicated that Th2 cells are endowed with as high a tyrosine phosphorylation capacity as Th1 cells. It therefore indirectly suggested that distinct phosphatases of higher activity or expressed at higher levels made Th2 cells less reactive than Th1 cells to TCR-mediated stimulation. To evaluate whether higher overall expression levels of phosphatases in Th2 than in Th1 cells might explain the distinct profiles of tyrosine phosphorylation in these two cell types, the relative amounts of CD45, SHP-1, and SHP-2 in Th1 and Th2 cells were assessed by Western blotting. Expression levels of these three tyrosine phosphatases were similar in Th1 and Th2 cells before and after TCR engagement (Fig. 6B). The absence of differences in total phosphatase expression levels between Th1 and Th2 cells suggested that the fainter phosphotyrosine profiles in Th2 than in Th1 cells might be alternatively explained by distinct enzymatic activities of phosphatases involved in the TCR signaling pathway or by distinct subcellular localization of phosphatases in the two Th subsets. The latter possibility was assessed by evaluating whether tyrosine phosphatases were excluded from lipid rafts to a comparable extent in Th1 and Th2 cells before TCR engagement (22). Th cell lysates were separated by sucrose gradient centrifugation, and the presence of phosphatases in the fractions was probed by Western blotting. CD45, SHP-1, and SHP-2 proteins were not detected in low density fractions, while the tyrosine kinase Lck was found in the low density material of Th1 and Th2 cells, indicating that membranes originating from lipid rafts centrifuged in these fractions (Fig. 6C). Collectively, these results indicated that, for the three abundant phosphatases analyzed and previously shown to be implicated in TCR-mediated signaling pathways, the fainter phosphotyrosine profiles in Th2 than in Th1 cells cannot be explained by distinct expression levels before and

FIGURE 5. CD28 and CD3 coengagement fails to restore tyrosine phosphorylation intensities in Th2 cells to levels obtained in Th1 cells. Following Th cell stimulation, lysates were prepared and electrophoresed as described. Tyrosine phosphorylation was revealed by Western blotting. Th1 and Th2 clones were stimulated for 2 min with 0 or 6 μg/ml anti-CD3 mAb plus 10 μg/ml goat anti-mouse IgG Ab in the presence or the absence of 2 μg/ml anti-CD28 mAb. Similar results were obtained in two independent experiments with three Th1 and three Th2 clones.
Stimulation was performed using 6 larsine oxide (c) and coimmunoprecipitated ZAP-70 protein was then evaluated by Western blotting in nonstimulated T cells fractionated on sucrose gradients. Lipid rafts were present in fraction 8, as revealed by the blots obtained in large quantities (300 mg). Since EBV-transformed B cells express high levels of DR protein, soluble peptide/DR complexes can be readily biochemically manipulated to obtain soluble, largely monomeric, Ag peptide/MHC class II complexes. The absence of detergent in this material made it suitable for cellular assays. In addition, this procedure offered the clear advantage that it did not require the development of expression vectors for each allelic form of MHC class II molecules. Since EBV-transformed B cells express high levels of DR protein, soluble peptide/DR complexes can be readily obtained in large quantities (300 μg DR protein/l EBV-B cell culture following this procedure). In contrast with MHC class II protein produced by recombinant techniques in insect cells or bacteria, MHC class II molecules isolated from B cells are not empty, but are occupied by cell- and medium-derived material. For that reason, it is expected that complete loading with Ag peptide cannot be attained, since, depending on the MHC class II alleles, occupancy with exogenously added peptides varies between <5% and up to 20–24%.

This study provided evidence that activation of Th1 cells was elicited at lower densities of immobilized peptide/MHC class II complexes, or anti-CD3 mAbs than that for Th2 cells. In contrast, Western blotting (Fig. 7). Following TCR engagement, phosphorylated ZAP-70 was associated with CD3ζ in activated Th1 cells. By contrast, in Th2 cells no ZAP-70 protein was detected in the material immunoprecipitated by anti-CD3ζ mAbs. In a control experiment using anti-TNF mAb instead of anti-CD3ζ mAb, no ZAP-70 was detected by Western blotting. Similarly, results were observed in three Th1 and three Th2 clones (data not shown). A lower association between CD3ζ and ZAP-70 in Th2 cells compared with Th1 cells by controlling the TCR signaling cascades might at least partly determine the overall fainter phosphorylation profiles in Th2 than in Th1 cells.

Discussion
The observations reported here constitute evidence that TCR signaling is distinct in human Th1 and Th2 cells. Analysis of up-regulation of the activation marker CD25 revealed that Th2 cells are less sensitive than Th1 cells upon TCR engagement with immobilized peptide/MHC class II complexes or anti-CD3 mAbs. This lower sensitivity of Th2 than Th1 cells was paralleled by an overall weaker tyrosine phosphorylation intensity in the former than in the latter cells. Distinct tyrosine phosphatase profiles might differentially control the Th1 and Th2 signaling cascades, as phenylarsine oxide treatment restored a high level of tyrosine phosphorylation in Th2 cells. Finally, TCR signaling in Th1 and Th2 differs at an early step of the cascade, as documented by a weaker association between CD3ζ and ZAP-70 in Th2 than in Th1 cells.

MHC class II protein preparations isolated from B cells were biochemically manipulated to obtain soluble, largely monomeric, Ag peptide/MHC class II complexes. The absence of detergent in this material made it suitable for cellular assays. In addition, this procedure offered the clear advantage that it did not require the development of expression vectors for each allelic form of MHC class II molecules. Since EBV-transformed B cells express high levels of DR protein, soluble peptide/DR complexes can be readily obtained in large quantities (300 μg DR protein/l EBV-B cell culture following this procedure). In contrast with MHC class II protein produced by recombinant techniques in insect cells or bacteria, MHC class II molecules isolated from B cells are not empty, but are occupied by cell- and medium-derived material. For that reason, it is expected that complete loading with Ag peptide cannot be attained, since, depending on the MHC class II alleles, occupancy with exogenously added peptides varies between <5% and up to 20–24%.

This study provided evidence that activation of Th1 cells was elicited at lower densities of immobilized peptide/MHC class II complexes, or anti-CD3 mAbs than that for Th2 cells. In contrast,
Th2 cells were as sensitive as Th1 cells upon TCR engagement with peptide/MHC class II complexes displayed by transfection at the surface of nonprofessional APC. These findings could be rationalized along two lines of reasoning. First, immobilized peptide/MHC class II complexes and anti-CD3 mAb cannot be clustered by T cells, while peptide/MHC class II molecules expressed on APC are laterally mobile and can be capped at the area of contact with T cells. Our observations might therefore suggest that Th2 cells require a greater degree of TCR clustering for activation than is the case for Th1 cells. Second, APC can provide Th2 cells with accessory signals needed for activation. DR1-transfected HeLa cells were used as a model of nonprofessional APC. It was assumed that these cells were largely devoid of secondary signaling molecules. In particular, they lack the expression of CD80, CD86, and CD40 molecules. In addition, it was found that CD3 and CD28 coengagement failed to increase tyrosine phosphorylation in Th2 cells to levels reached by Th1 cells. These results indicated that the lower activation ability of Th2 than of Th1 cells was intrinsic to the TCR signaling cascades, rather than due to the absence of CD28 costimulatory signals. Together, our results favored the idea that activation of Th2 cells might require more robust TCR-mediated signals than is the case for Th1 cells. Interestingly, it was recently shown in the murine system that TCR complexes of Th1 cells are efficiently recruited into rafts at the area of contact with peptide/MHC class II complexes, while such marked TCR partitioning does not occur in Th2 cells (26). Our results might suggest that these differences in plasma membrane dynamics are also found between human Th1 and Th2 cells.

The differential reactivity of human Th1 and Th2 cells observed in this study could be explained by distinct features in the proximal events of the TCR signaling machinery, as revealed by a weaker association between CD3ζ and ZAP-70 in human Th2 than in Th1 cells. In the mouse model a looser interaction between CD3ζ and ZAP-70 in Th2 than in Th1 cells was similarly reported by Tamura et al. (15). These authors suggested that the lower activities of Fyn and ZAP-70 observed in Th2 cells could explain this weaker association. As postulated in the mouse system, the weaker association between CD3ζ and ZAP-70 in Th2 than in Th1 cells might be due to a weaker activity of Fyn and ZAP-70 kinases. Interestingly, the activities of Fyn and of ZAP-70 kinases were reduced in human Th cells activated by altered peptide ligands promoting Th2-like phenotypes (27). In contrast, a previous study showed that in differentiated human Th cells the two subsets do not differ in the overall tyrosine phosphorylation profiles, particularly in tyrosine phosphorylation of ZAP-70 (16). Different procedures for the isolation of human Th1 and Th2 cells and the resulting distinct phenotypes of these cells might explain the apparent discrepancies between these studies.

Tyrosine phosphatases have been shown to play a key role in the early steps of TCR signaling (28). In particular, CD45 was shown to activate Lck, a protein kinase involved in the first steps of the TCR signaling cascades (29, 30). To identify which phosphatases might be responsible for the faint phosphotyrosine profiles in Th2 cells, expression levels of CD45, SHP-1, and SHP-2 were assessed in Th1 and Th2 cells. Comparable amounts of these three phosphatases were detected in lysates of Th1 and Th2 cells. Moreover, CD45, SHP-1, and SHP-2 could not be detected in lipid rafts of both Th subtypes before TCR engagement. These observations indicated that before TCR engagement, neither the overall expression level nor the subcellular localization of these phosphatases could account for the reduced phosphotyrosine profiles in Th2 compared with Th1 cells. Interestingly, it was recently shown that CD45 was recruited in lipid rafts after TCR engagement in mouse Th1, but not Th2, cells (26). It may be postulated that CD45 recruitment also occurs in human Th1, but not Th2, cells after TCR engagement. In Th2 cells the absence of clustering of TCR and CD45 in membrane domains involved in signal transduction might result in the failure of efficiently initiating the TCR signaling cascades. Nevertheless, it cannot be excluded that other phosphatases could be involved in the distinct TCR signaling events found in Th1 and Th2 cells. It was suggested that phosphatases other than CD45, SHP-1, and SHP-2 are present in the T cell plasma membrane (31). Moreover, distinct phosphatase expression patterns were reported in Th1 and Th2 subsets. While CD45β was detected in murine Th1, but not in Th2, clones (32), Fas-associated phosphatase 1 (FAP-1) mRNA was expressed at high levels in Th2, but not in Th1, cells (33). It was suggested that FAP-1, a membrane tyrosine phosphatase, is involved in resistance to activation-induced cell death in Th2 cells, suggesting a role for FAP-1 in TCR signaling cascades.

Identification and characterization of molecules such as phosphatases that differentially control early steps of TCR signaling cascades in Th1 and Th2 cells might provide new avenues for the pharmacological manipulation of deregulated Th cell functions. Future work would aim at evaluating whether interfering with early steps of the TCR signaling cascades distinguishing Th1 and Th2 cells might result in a beneficial impact on various immune-related pathological manifestations, such as autoimmune diseases and allergic reactions.

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References


