A Role for the Cytoplasmic Tail of the Pre-T Cell Receptor (TCR) α Chain in Promoting Constitutive Internalization and Degradation of the Pre-TCR

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During intrathymic development of αβ T cells, thymocytes that have a successful rearrangement at the T cell receptor β (TCRβ) locus express a pre-TCR complex composed of the TCRβ chain paired with the invariant pre-TCRα (pTα) chain and associated with CD3 components (1–3). Surface expression of this pre-TCR (4) triggers the selection, expansion, and further differentiation of developing pre-T cells in a ligand-independent manner (5, 6), finally resulting in the induction of rearrangements at the TCRα locus. Upon productive TCRα gene rearrangements, the TCRα chain pairs with TCRβ and associates with CD3ε, γ-δ, and ζ chains, and thymocytes undergo a second step of selection upon binding of the TCRβ to self-peptide-major histocompatibility complex molecules (1–3). Despite experimental evidence on the similar biochemical compositions of the pre-TCR and TCR in terms of their associated CD3 subunits (7–9), current studies support the theory that mechanisms regulating the assembly and intracellular transport of these complexes may differ markedly, because the pre-TCR is expressed only transiently and very inefficiently during thymocyte development, at levels about 50–100-fold lower than those of the TCR on mature T cells (3, 10). By using TCRα-pTα chain chimeras, we have shown recently (9) that limited expression of the human pre-TCR is pTα chain-dependent. Particularly, the pTα cytoplasmic (Cy) domain was found to serve an endoplasmic reticulum retention function that could contribute in part to the regulation of pre-TCR assembly and surface expression (9).

However, the level of expression of a cell surface receptor is the result of an equilibrium between the synthesis and transport of new polypeptides and their internalization, recycling, and degradation (reviewed in Ref. 11). Extracellular stimuli induce changes in one or several of these processes and therefore modify the level of expression of a given receptor. In the particular case of the TCR, receptor engagement by its natural ligands results in down-regulation of TCR-CD3 cell surface expression (11–14), which is a critical event intimately associated with TCR signaling and T-cell activation (14). Different molecular mechanisms have been proposed to account for the down-modulation of ligated TCR complexes. Most studies support the position that TCR ligation results in a significant increase of the TCR internalization rate followed by the degradation of the internalized complex (15, 16). However, TCR-CD3 complexes are continuously internalized and recycled back to the cell surface in nonstimulated T cells (17, 18), suggesting that constitutive TCR recycling on resting T cells has to be affected by TCR ligation. In this regard, Wiest and co-workers (19) have recently proposed that TCR engagement has little effect on the TCR internalization rate; rather, it prevents TCR recycling back to the cell surface by inducing the intracellular retention of ligated complexes and their degradation by lysosomes and proteasomes.

In sharp contrast to the TCR, pre-TCR signaling occurs apparently without any need for ligation (5, 6). This concurs with the finding that the pre-TCR spontaneously clusters and localizes on the cell surface into lipid rafts (20, 21), in a manner similar to that found for the αβ TCR following ligand binding (22, 23). These findings support the view that surface pre-TCR...
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complexes are constitutively activated without any need for ligation (20) and raise the question of what is the intracellular fate of such “activated” pre-TCR complexes. In this study, we have analyzed the dynamics of pre-TCR-CD3 cell surface expression and down-modulation in unstimulated pre-T cells and showed that, similar to ligated TCR complexes, surface pre-TCR complexes are continually and rapidly endocytosed and degraded in the absence of extracellular ligation but do not recycle back to the cell surface. Moreover, we show that cell-autonomous or pre-TCR down-modulation is dependent on pTcytoplasmic tail. The possibility that constitutive endocytosis and degradation of the pre-TCR is a self-safe mechanism responsible for its limited expression on the cell surface is discussed.

EXPERIMENTAL PROCEDURES

Cell Lines and Transfectants—The α and α/CypTα stable transfectants were derived as described elsewhere (9) from the pre-T cell line SUP-T1, which expresses an endogenous TCRβ (Vβ1) chain and the pTα chain but lacks TCRα. Briefly, SUP-T1 cells were transfected, respectively, either with full-length cDNAs encoding a conventional TCRα (Vα12.1) chain or with a TCRα/pTα chimeric construct in which the Cy domain of TCRα was replaced by the equivalent domain of pTα (CypTα). G418-selected transfected cells were grown in RPMI 1640 (Bio-Whittaker) supplemented with 10% fetal calf serum (Invitrogen). Likewise, TCR-α/GFP stable transfectants were derived from SUP-T1 cells transfected with a plasmid encoding a COOH-terminal fusion protein of the TCRα chain with the green fluorescence protein (GFP). The TCR- α/GFP fusion was performed by PCR amplification of a complete TCRα chain cDNA (kindly provided by Dr. B. Alarcon, Centro de Biología Molecular Severo Ochoa, Madrid, Spain) with the sense 5′-GGC GGC CCC GGG ATG TGG AAG GGC CCT TCT-3′ and antisense 5′-GGG CCG GCC GGC CCC AGG AGG GAG GGC-3′ primers followed by digestion of cytoplasmic tail. The possibility that constitutive endocytosis and degradation of the pre-TCR is a self-safe mechanism responsible for its limited expression on the cell surface is discussed.

Internalization and Recycling Assays—SUP-T1 cells and α/CypTα, and TCR-α/GFP stable transfectants were cultured (5–8 × 10^6) in 24-well plates (Costar) either in the absence or presence of cycloheximide (10 ng/ml, Calbiochem), brefeldin-A (1 μg/ml, Sigma), and/or PP2 (2 μM, Calbiochem). At different time points, surface expression of the endogenous pre-TCR was analyzed by flow cytometry (EPICS XL Coulter Corp. with phycocerythrin-labeled anti-human TCRβ1 (Endogen) or anti-CD3 (BD Biosciences) mAbs). Surface levels of the conventional TCRα-β (TCR) or the chimeric TCRα/CypTα-β (ChTCR) receptors respectively were assayed on α/CypTα transfectants was analyzed with the anti-TCRβ mAb BMA031 (Behring-Werke AG) and G418 transfected with an anti-CD3 mAb and immunoprecipitated proteins (Clontech). Pre-TCR surface expression levels analyzed on 48 selected TCR-α/GFP clones were similar to those on parental SUP-T1 cells.

Surface Pre-TCR-CD3 Complexes Are Continually Internalized but Do Not Recycle Back to the Cell Surface—To study the dynamics of pre-TCR cell surface expression, we used a human pre-T cell line, SUP-T1, which has been shown to display low pre-TCR surface levels as found on primary human pre-T cells (Ref. 9 and Fig. 1A). The relative contribution of synthesis and secretion of new chains as compared with internalization and recycling of expressed ones to actual pre-TCR-CD3 surface levels was explored by comparative cycloheximide or brefeldin-A (BFA) treatment, as described recently for the mature TCR-CD3 complex (19). Pre-TCR surface expression was measured with an anti-TCRβ1 mAb (9). The dynamics of TCR-CD3 surface expression was analyzed for comparison in the same cellular environment, namely SUP-T1 cells stably transfected with a TCRα chain (α/CypTα transfectants), which co-expressed the conventional TCRαβ-CD3 complex (>99% TCRαβ) together with the endogenous pre-TCR (Ref. 9, and Fig. 1A). The results confirmed that, as described (19), the mature surface TCR-CD3 was relatively synthesis-independent, that is, surface TCR-CD3 expression levels remained essentially stable within the studied 12-h time period (Fig. 1C). Therefore, mature TCR complexes are long-lived on α/CypTα transfectants. As reported (19), the effect of BFA on TCR expression was very different from that of cycloheximide and resulted in a partial reduction of TCR membrane levels (Fig. 1A), which fell rapidly during the first 2 h of treatment (about 30% reduction) and remained low (50% of control levels) for 8 h thereafter (Fig. 1B). Because surface expression of the mature TCR was found independent of newly synthesized complexes, this reduction in TCR surface levels cannot be due to the reported BFA-induced blockade of the anterograde transport from the endoplasmic reticulum to the Golgi complex (24, 25). Rather, it may be caused by the documented capacity of BFA to induce tubulation and fusion of the trans-Golgi network with early endosomes, which, although previously reported to leave cycling between plasma membrane and endosomes of certain molecules such as transferrin unperturbed (24), has recently been shown to affect the endocytic transport of the TCR (19, 26). Therefore, our data
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The effects of BFA and cycloheximide on surface expression of the pTα cytoplasmic domain are constitutively internalized but do not recycle back to the cell surface. A, SUP-T1 untransfected cells or cells stably transfected with a TCRα (wt) or with a chimeric α/CypTα chain (α/CypTα) were treated for 6 h with cycloheximide (thin line) or brefeldin-A (thick line) or were left untreated (shaded histogram). Pre-TCR surface expression on SUP-T1 cells was determined by flow cytometry with a phycoerythrin-labeled mAb against the endogenous TCRβ (Vγ1) chain. Surface expression of TCR and ChTCR complexes on α/CypTα transfectants, respectively, was assessed with the BMA031 mAb against monomorphic determinants of human TCRβ heterodimer. The effect of brefeldin-A (B) and cycloheximide (C) on the expression levels of the endogenous pre-TCR on SUP-T1 cells, the TCRαβ on α/CypTα transfectants, and the ChTCR on α/CypTα transfectants was analyzed by flow cytometry at the indicated time points, as described in A. The percentage of surface expression was determined from the mean fluorescence values of treated cells, using the untreated controls as reference. Results are representative of four independent experiments.

FIG. 1. Surface pre-TCR and chimeric TCR-pTα (ChTCR) complexes containing the pTα cytoplasmic domain are constitutively internalized but do not recycle back to the cell surface. A, SUP-T1 untransfected cells or cells stably transfected with a TCRα (wt) or with a chimeric α/CypTα chain (α/CypTα) were treated for 6 h with cycloheximide (thin line) or brefeldin-A (thick line) or were left untreated (shaded histogram). Pre-TCR surface expression on SUP-T1 cells was determined by flow cytometry with a phycoerythrin-labeled mAb against the endogenous TCRβ (Vγ1) chain. Surface expression of TCR and ChTCR complexes on α/CypTα transfectants, respectively, was assessed with the BMA031 mAb against monomorphic determinants of human TCRβ heterodimer. The effect of brefeldin-A (B) and cycloheximide (C) on the expression levels of the endogenous pre-TCR on SUP-T1 cells, the TCRαβ on α/CypTα transfectants, and the ChTCR on α/CypTα transfectants was analyzed by flow cytometry at the indicated time points, as described in A. The percentage of surface expression was determined from the mean fluorescence values of treated cells, using the untreated controls as reference. Results are representative of four independent experiments.

In contrast, pre-TCR down-modulation was coupled with a dramatic increase of green fluorescence in BFA-treated cells, indicating that ζ-GFP protein chimeras had accumulated in the cytoplasm. More importantly, blocking of endosome to lysosome trafficking induced by BFA (24) counteracted the effect of cycloheximide and prevented the disappearance of GFP expression, which remained stable although partly reduced (≥60% of control levels) after 12 h of treatment with cycloheximide (Fig. 2B), suggesting that the ζ-GFP chimeric chains, including those associated with the internalized pre-TCR, were degraded intracellularly. In contrast, pre-TCR down-modulation was coupled with a dramatic increase of green fluorescence in BFA-treated cells, indicating that ζ-GFP protein chimeras had accumulated in the cytoplasm. More importantly, blocking of endosome to lysosome trafficking induced by BFA (24) counteracted the effect of cycloheximide and prevented the disappearance of GFP expression, which remained stable although partly reduced (≥60% of control levels) after 12 h of treatment with cycloheximide (Fig. 2B), suggesting that the ζ-GFP chimeric chains, including those associated with the internalized pre-TCR, were degraded intracellularly.

Supporting this possibility, confocal microscopy analysis showed that untreated ζ-GFP transfectants had low levels of surface ζ-GFP that colocalized with the membrane marker CD4 (Fig. 2C), whereas a significant amount of green fluorescence was expressed intracellularly and accumulated in the cytoplasmic structures that expressed the lysosomal marker CD63 (27). As shown in Fig. 2D a high proportion of ζ-GFP (55 ± 17%) colocalized with CD63 on different 0.2 μm sections. According to flow cytometry data, ζ-GFP expression was sensitive to BFA treatment, so that a significant increase of green fluorescence was observed in BFA-treated cells in which ζ-GFP accumulated intracellularly and acquired a characteristic distribution in tubular structures typical of BFA-treated cells (Fig. 2E). Expectedly, no ζ-GFP expression was detected upon cycloheximide treatment (data not shown), again supporting the possibility that pre-TCR-associated intracellular TCRζ was degraded. However, intracellular accumulation of ζ-GFP was observed when cells were treated simultaneously with cycloheximide and BFA (Fig. 2F), indicating that degradation of cytoplasmic ζ-GFP was blocked because of the impaired trafficking to lysosomes induced by BFA.

It has been shown that the primary mechanism mediating down-modulation of the TCR upon ligand binding involves...
targeting of endocytosed complexes for intracellular degradation, predominantly by lysosomes, but also by proteasomes (16, 19). To investigate whether proteasome was also involved in degradation of endocytosed pre-TCR or ChTCR complexes, biochemical studies were performed aimed at analyzing the intracellular fate of TCRβ associated to internalized complexes in cells treated with drugs that block lysosome (NH₄Cl and chloroquine) or proteasome (lactacystin) function. None of these drugs affected surface receptor expression levels or cellular viability (data not shown). However, as shown by immunoblot analysis (Fig. 3A), they induced a significant accumulation of intracellular TCRβ dimers that were immunoprecipitated as associated to the pre-TCR or the ChTCR from SUP-T1 cells or α/CypTα transfectants, respectively. In contrast, these drugs did not significantly affect the levels of TCRβ chain associated to the mature TCR complex in αwt transfectants, which is consistent with an active and selective degradation of TCRβ dimers associated with the pre-TCR and the ChTCR but not with the conventional TCR. Accordingly, degradation of TCRβ dimers associated to pre-TCR and ChTCR complexes was observed when protein synthesis was blocked with cycloheximide (Fig. 3A), but TCRβ chain degradation was inhibited by NH₄Cl and chloroquine, and to a lesser extent, by lactacystin (see densitometric analysis in Fig. 3B). Taken together, our data provide evidence that, as shown previously for ligated TCR CD3 complexes, constitutive internalization of unligated pre-TCR-CD3 complexes is followed immediately by TCRβ chain degradation, mainly by lysosomes, but also by proteasomes, which prevents recycling to the cell surface. Moreover, they suggest that the pTα Cy tail is involved selectively in that process.

The pTα Cy Domain Is Sufficient to Divert the TCRα-TCRβ Heterodimer from a Recycling Pathway to Intracellular Degradation—To determine whether, as shown for TCRβ dimers, internalized pTα-TCRβ heterodimers are targeted for intracellular degradation, we next analyzed biochemically the intracellular fate of biotin-labeled surface pre-TCR-CD3 complexes. Immunoblot analysis with avidin-peroxidase of anti-CD3e immunoprecipitates confirmed that the pTα-TCRβ heterodimer was rapidly degraded in SUP-T1 pre-T cells. As shown in Fig. 4A, 40% of the biotinylated heterodimers had disappeared after 90 min, and less than 30% of the input pTα-TCRβ complexes remained after 4 h, as assessed by densitometric analysis (Fig.
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4B). In contrast, no degradation of the mature TCRαβ heterodimer was observed in αwt transfectants, in which biotinylated complexes remained stable for 4 h. Strikingly, heterodimers composed of the TCRβ chain bound to the chimeric α/CypTα chain behaved essentially as pTα-TCRβ heterodimers. Moreover, the simultaneous analysis of the ChTCR and the endogenous pre-TCR coexpressed in α/CypTα transfectants revealed that the total content of these two heterodimers decreased with identical kinetics (Fig. 4B), demonstrating that the pTα Cy tail is sufficient to determine the degradation fate of the pre-TCR components.

To investigate the intracellular degradation pathway followed by the pTα-TCRβ heterodimer, we next performed immunoblot analysis of anti-CD3ε immunoprecipitates from biotin-labeled SUP-T1 pre-T cells treated with drugs that affect either the lysosome (chloroquine) or proteasome (lactacystin and epoxomicin) function. Blotting with anti-α-tubulin was used as an internal control of protein loading (Fig. 5A). These studies revealed that about 70% of the biotinylated pTα-TCRβ heterodimers was lost after a 6-h chase as assessed by densitometric analysis (Fig. 5B). Surprisingly, a complete inhibition of the degradation of pTα-TCRβ heterodimers was observed in cells treated with lactacystin, an degradation was likewise sensitive to epoxomicin, two specific and irreversible proteasome inhibitors, whereas a weak inhibitory effect was observed after a 6-h chase in the presence of the lysosome inhibitor chloroquine. Therefore, although these data can not rule out the possibility that a proportion of the pTα-TCRβ heterodimers is degraded in lysosomes, they are consistent with a prominent role for the proteasome in the constitutive degradation of the internalized pTα-TCRβ heterodimers.

Molecular Mechanisms Involved in Constitutive Pre-TCR Down-regulation—Phosphorylation of the cytoplasmic tail of CD3γ by PKC is the mechanism responsible for constitutive TCR internalization in unstimulated T cells (28). Therefore, we analyzed whether constitutive pre-TCR internalization in unstimulated pre-T cells is also PKC-dependent. However, no effects on surface pre-TCR levels were observed after treatment of SUP-T1 cells with doses of phorbol 12-myristate 13-acetate, which induced 60% down-regulation of the mature TCR in αwt transfectants. Neither was ChTCR down-modulation induced by phorbol 12-myristate 13-acetate in α/CypTα transfectants even after 2 h of treatment (Fig. 6A). These data, together with the fact that bisindolylmaleimide and Ro-31–7549, two specific PKC inhibitors, did not affect pre-TCR surface expression (data not shown), suggest that PKC is not involved in the constitutive internalization of the pre-TCR.

An intriguing possibility is that protein tyrosine kinases (PTK) such as Lck, which might play a key role in cell-autonomous signaling through the pre-TCR (20) could participate in its down-regulation, as shown for engaged TCR-CD3 complexes (29). To address this possibility, we analyzed the effects of the src family (Lck/Fyn) PTK inhibitor PP2 on surface pre-TCR expression. As shown in Fig. 6B, inhibition of src kinases resulted in increased expression levels of the pre-TCR and the ChTCR in SUP-T1 and α/CypTα transfectants, respectively. In contrast, PP2 treatment had no effect on the expression levels of the mature TCR in Jurkat T cells (data not shown), although some increase in TCR expression was observed in αwt transfectants. To assess whether the increase in surface pre-TCR levels upon Lck/Fyn inhibition could represent a blockade in
both pre-TCR internalization and degradation, we next analyzed the kinetics of pre-TCR down-regulation in cycloheximide-treated SUP-T1 pre-T cells, with or without PP2. As shown in Fig. 6C, inhibition of src kinases consistently delayed, but could not block, the constitutive internalization and down-regulation of the pre-TCR. Therefore, mechanisms involving phosphorylation by src kinases are partially, but not fully, responsible for constitutive pre-TCR down-regulation.

**DISCUSSION**

In this study, comparative analyses on the dynamics of human pre-TCR and TCR cell surface expression and down-modulation revealed striking differences in the behavior and intracellular fate of unligated TCR and pre-TCR complexes. We have shown that TCR-CD3 complexes expressed on SUP-T1 cells, upon transfection with TCRζ, are constitutively internalized and recycled back to the cell surface in the absence of ligand binding, and thus behave as conventional unligated TCR complexes on resting mature T cells (17–19). In contrast, pre-TCR complexes expressed on unstimulated SUP-T1 pre-T cells are continually and rapidly endocytosed but do not recycle back to the cell surface. As reported for TCR complexes internalized following antigen stimulation (16, 19), we show here that intracellular degradation is the mechanism responsible for the impaired recycling of pre-TCR in unstimulated pre-T cells. Strikingly, we found that ζ chain dimers associated to the internalized pre-TCR are sorted for degradation in lysosomes and proteasomes and thus follow the intracellular fate of TCR-ζ chain complexes internalized following antigen stimulation (16, 19), whereas evidence is provided that the proteasome plays a prominent role in the constitutive degradation of the internalized pTα-TCRβ heterodimers. In this regard, it is worth noting that, as observed upon TCR ligation, internalized ζ chains associated with the unligated pre-TCR are found mostly in a phosphorylated state, a characteristic event associated with T cell activation (30). Therefore, the human pre-TCR complex behaves constitutively as an activated TCR without any need for ligand binding. After submission of this manuscript, Panigada et al. (31) provided evidence of constitutive pre-TCR internalization and degradation in the mouse. Our results extend the peculiar behavior of the pre-TCR to the human, and map it to the cytoplasmic tail of the pTα chain by comparison with TCRζ, which does not share this capacity.

Ligand-independent activation of the pre-TCR has recently been proposed to result from its constitutive co-localization in membrane rafts with signaling molecules, such as Lck (20), which may trigger cell-autonomous activation of proximal signaling including CD3ε and Zap70 phosphorylation in a manner similar to that observed for the ligated αβ TCR (22, 23). Such a particular membrane distribution might depend on the unique biochemical structure of the pre-TCR. Particularly, it was proposed that palmitoylation of the conserved juxta-membranous cysteine residue of the pTα Cy domain might be required for the cell-autonomous raft localization of the pre-TCR (20). However, a very recent study has proved that this is not an essential component for endowing the murine pre-TCR with cell-autonomous signaling capability (32). Although this finding might support the current view that the Cy domain of the murine pTα molecule is dispensable for pre-TCR function, the same study provides direct evidence that the COOH-terminal proline-rich domain of the murine pTα Cy tail plays a crucial role in pre-TCR signaling and T cell development (32). This finding supports our proposal that the Cy tail of the human pTα molecule is an essential component of pre-TCR function (9) and concurs with the present finding that the pTα Cy tail is sufficient to confer constitutive internalization and degradation properties to the conventional TCR. It is thus likely that the same mechanisms involved in ligand-induced TCR signaling and down-regulation could control the pre-TCR-CD3 intra-cellular fate. Accordingly, phosphorylation of CD3γ by PKC, which is currently believed to control the internalization and recycling of unligated TCR complexes but not ligand-induced TCR down-modulation (11, 28), does not seem to play a role in pre-TCR down-modulation. Regarding the potential role of PTKs involved in TCR signaling such as Lck and Fyn in TCR down-regulation, the available data support the view that down-regulation of engaged TCR complexes involve both PTK-dependent and -independent mechanisms, which are most likely controlled by the concentration of ligand and final receptor occupancy (11, 13, 30). Because pre-TCR internalization was found to be only partly dependent on src kinase activity, the situation may be equivalent to that reported for the TCR at maximal receptor occupancy (13).

Alternatively, it can be proposed that constitutive internalization and degradation of the pre-TCR depends on unique endocytosis and/or degradation motifs. In this regard, the CD3 and TCRζ components shared by pre-TCR and TCR display internalization/sorting motifs of both the dileucine- and the tyrosine-based types, which could mediate clathrin-dependent internalization and intracellular sorting to degradation (reviewed in Ref. 11). Interestingly, a consensus tyrosine-based motif (*226YPTC229*) exists within the Cy domain of the human pTα molecule as well, which could become cell-autonomously exposed in the activated pre-TCR conformation to fulfill both the internalization and degradation functions. It is also possible that association of the pre-TCR into lipid rafts could regulate a constitutive clathrin-independent endocytic pathway similar to that recently described for the interleukin-2 receptor (33). Whatever the mechanism involved, we would suggest that constitutive internalization and degradation of the pre-TCR is a key process that controls surface receptor levels and provides the cell with a self-safe mechanism to avoid sustained ligand-independent signaling through a potent, potentially oncogenic, cell growth receptor (34).
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REFERENCES
1. von Boehmer, H., and Fehling, H. J. (1997) Annu. Rev. Immunol. 15, 433–452
2. Malissen, B., Ardoniu, L., Lin, S.-Y., Gillet, A., and Malissen, M. (1999) Adv. Immunol. 72, 103–148
3. Borst, J., Jacobs, H., and Brouns, G. (1996) Curr. Opin. Immunol. 8, 181–190
4. O’Shea, C. C., Thornel, A. P., Rosewell, I. R., Hayes, B., and Owen, M. J. (1997) Immunity 7, 591–599
5. Jacobs, H., Iacomini, J., van de Ven, M., Tonegawa, S., and Berns, A. (1996) J. Exp. Med. 184, 1833–1843
6. Irving, B. A., Alt, F. W., and Killeen, N. (1998) Science 280, 965–968
7. Berger, M. A., Dave, V., Rhodes, M. R., Bosma, G. C., Bosma, M. J., Kappes, D. J., and Wiest, D. L. (1997) J. Exp. Med. 186, 1461–1467
8. Van Oers, N. S. C., von Boehmer, H., and Weiss, A. (1995) J. Exp. Med. 182, 1585–1595
9. Carrasco, Y. R., Ramiro, A. R., Trigueros, C., de Yebenes, V. G., Garcia-Peydró, M., and Toribio, M. L. (2001) J. Exp. Med. 193, 1045–1057
10. Trigueros, C., Ramiro, A. R., Carrasco, Y. R., de Yebenes, V. G., Albar, J. P., and Toribio, M. L. (1998) J. Exp. Med. 188, 1401–1412
11. Alcover, A., and Alarcon, B. (2000) Crit. Rev. Immunol. 20, 325–346
12. Zanders E. D., Lamb, J. R., Feldmann, M., Green, N., and Beverley, P. C. (1983) Nature 302, 625–627
13. Cai, Z., Kishimoto, H., Brunmark, A., Jacksen, M. R., Peterson, P. A., and Sprent, J. (1997) J. Exp. Med. 185, 641–654
14. Valtiutti, S., Muller, S., Celli, M., Padovan, E., and Lanzavecchia, A. (1995) Nature 375, 148–150
15. Niedergang, F., Hemar, A., Hewitt, C. R., Owen, M. J., Dautry-Varsat, A., and Alcover, A. (1995) J. Biol. Chem. 270, 12839–12845
16. Valtiutti, S., Muller, S., Salio, M., and Lanzavecchia, A. (1997) J. Exp. Med. 185, 1859–1864
17. Krangel, M. S. (1987) J. Exp. Med. 165, 1141–1159
18. Minami, Y., Samelson, L. E., and Klausner, R. D. (1987) J. Biol. Chem. 262, 13342–13347
19. Liu, H., Rhodes, M., Wiest, D. L., and Vignali, D. A. A. (2000) Immunity 13, 665–675
20. Saint-Ruf, C., Panigada, M., Azogui, O., Dekey, P., von Boehmer, H., and Grasso, F. (2000) Nature 406, 524–527
21. Ramiro, A. R., Navarro, M., Carreza, A., Carrasco, Y. R., de Yebenes, V. G., Carrillo, G., San Millán, J. L., Rubin, B., and Toribio, M. L. (2001) J. Immunol. 167, 5106–5114
22. Janes, P. W., Ley, S. C., and Magee, A. I. (1989) J. Cell Biol. 147, 447–461
23. Monti, C., Langet, C., Bernard, A.-M., Thimonier, J., Dubois, C., Wurbel, M.-A., Chauvin, J.-P., Pierres, M., and He, H.-T. (1998). EMBO J. 17, 5334–5348
24. Lippincott-Schwartz, J., Yuan, L., Tipper, C., Amherdt, M., Orci, L., and Klausner, A. D. (1991) Cell 67, 601–616
25. Wood, S. A., Park, J. E., and Brown, W. J. (1991) Cell 67, 591–600
26. van Essen, M., Menne, C., Nielsen, B. L., Lauritsen, J. P. H., Dietrich, J., Andersen, P. S., Karjalainen, K., Odum, N., and Geisler, C. (2002) J. Immunol. 168, 4519–4525
27. Metzelaar, M. J., Wijngaard, P. L., Peters, P. J., Sixma, J. J., Nieuwenhuis, H. K., and Clevers, H. C. (1991) J. Biol. Chem. 266, 3239–3245
28. Dietrich, J., Hsu, W., Wegener, A. M., and Geisler, C. (1994) EMBO J. 13, 2156–2166
29. D’Oros, U., Vacchio, M. S., Weissman, A. M., and Ashwell, J. D. (1997) Immunity 7, 619–628
30. Itoh, Y., Hemmer, B., Martin, R., and Germain, R. N. (1999) J. Immunol. 162, 2973–2980
31. Panigada, M., Porcellini, S., Barbier, E., Hoeflinger, S., Cazenave, P.-A., Gu, H., Band, H., von Boehmer, H., and Grasso, F. J. Exp. Med. 195, 1585–1597
32. Aifantis I, Borowski Ch., Gounari, F., Lacorazza H. D., Nikolich-Zugich, J., and Rosen, D. J., and Wiest, D. L. (1997) J. Exp. Med. 185, 1859–1864
33. Lamaze, C., Dujeancourt, A., Babu, T., Lo, C. G., Benmerah, A., and Dautry-Varsat, A. (2001) Mol. Cell 7, 661–671
34. Jacobs, H, Ossendorp, F, de Vries, E, Ungewiss, K, von Boehmer, H, Borst, J, and Berns, A. (1996) Oncogene. 12, 2089–2099
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