THE ROLE OF THE ZETA CHAIN IN THE EXPRESSION, STRUCTURE AND FUNCTION OF THE T CELL RECEPTOR

Richard D. Klausner, Allan M. Weissman, Michal Baniyash, Juan S. Bonifacino and Lawrence E. Samelson

Mechanisms of Lymphocyte Activation and Immune Regulation II pp 21-24|

INTRODUCTION

The T Cell Antigen Receptor (TCR) is an extremely complex cell surface receptor that plays a key role in both the development and mature function of the immune system serving the dual functions of antigen recognition and transmembrane signaling1,2. The overall structure of the receptor and how it gene rates appropriate physiologie trans membrane signals is the object of studies in our laboatory. Two types of components make up the TCR: I) clonotypic chains (Ti alpha and beta or Ti gamma and delta) provide the recognition function and antigen-MHC specificity of the receptor; and 2) non-polymorphie chains (CD3) most likely determine the signalling capacity of the receptor. The receptor is defined structurally by the non-covalent assemblage of a clonotypic heterodimer with the complete set of CD3 chains. This requisite co-assembly is underscored by the fact that the lack of any of the above components leads to a deficiency in the surface expression of the multichain complex. The most recently characterized component of the receptor complex is the zeta chain3- S. This was first observed in murine and subsequently found in human T cells. Zeta is a 16 kD protein as determined by migration on SDS-PAGE. It is found only in T cells and exists primarily as a homodimer. Its pI of 8.2-8.3 makes it the most basic of all of the components of the surface TCR and it carries no N-linked carbohydrate as determined by both metabolie labeling and treatment with endoglycosaminidases.

MOLECULAR CLONING OF THE ZETA

cDNA Purification of murine zeta chains allowed for the production of anti-zeta antibodies which recognize no other TCR components and, due to cross reaction, allowed for the initial identification of the human zeta. Further, purification of immunoprecipitated zeta on non-reducingjreducing PAGE gels allowed for protein sequencing of mature zeta to be carried out. Based on pro tein sequence, oligonucleotide probes were designed and used to isolate cDNA's encoding the murine zeta chain6. We have used the zeta cDNA to isolate the corresponding human cDNA and murine and human genes. The murine zeta chain is encoded by a single gene which produces a 1.7-1.8 kb message. This mRNA in only found in T cells. A 1.2 kb cDNA which encodes the zeta protein was sequenced. A single open reading frame predicts a 164 amino acid protein with a molecular wright of 18,637. Hydrophobicity analysis demonstrates an amino terminal hydrophobie region suggestive of a leader peptide and a single additional hydrophobie stretch compatable with a transmembrane helix7. Further analysis of the amino terminus by the method of von Heijne strongly points to this region being a 21 S. Gupta et al. (eds.), Mechanisms of Lymphocyte Activation and Immune Regulation II leader peptide with the predicted site of c1eavage between amino acids 21 and 228• Such a c1eavage would result in a mature protein whose 143 residues has a molecular weight of 16,299. A model of zeta reveals an unusual transmembrane protein. It contains only 9 extracellular amino acids followed by a 21 amino acid transmembrane segment. The carboxy terminal 1 I3 amino acids

form a long cytoplasmic tai!. No nucleotide or amino acid sequence similarity was observed between zeta and any of the other components of the TCR or to any other known protein. One interesting feature is shared by zeta and all of the other TCR components. The predicted transmembrane region of zeta contains a single negative charge. All of the other CD3 components similarly possess a negatively charged residue in their predicted transmembrane domains9- 11. The c1onotypic components contain positively charged residues in these hydrophobic domains12- 14. The presence of charges in these regions is a very unusual feature for transmembrane proteins. A single cysteine at the external face of the membrane spanning domain defines the site of disulfide bond formation. Finally six intracytoplasmic tyrosines are potential sites for the phosphorylation of zeta that is seen upon receptor activation. One additional feature of zeta revealed by the sequence is a possible binding site for A TP due to the presence of a consensus sequence (Gly-x-Gly-x-x-Gly-x-x-x-Gly ... Ala-x-Lys) first recognized in protein kinases and which is found at the carboxy terminus of the zeta protein15• In vitro translation of RNA transcripts made from the cloned cDNA resulted in the production of an 18.5 kD protein that was immunoprecipitated by two different anti-zeta antisera. When translation was carried out in the presence of dog pancreas microsomes the 18.5 kD protein was largely processed to a 16 kD form consistent with SDS-PAGE migration of mature T cell zeta and again, was specifically recognized by anti-zeta antibodies. An antibody raised in rabbits against a peptide present in the predicted sequence, recognized zeta in murine T cells further confirming the identity of the clone. The murine cDNA clone was used to screen a human cDNA Iibrary derived from T cells. The human zeta clone recognized a 1.9 to 2.0 kb mRNA expressed only in T cells. Sequencing of the human cDNA showed a very high degree of conservation in the protein coding region (85% nucleotide, 87% amino acid). All of the following features are conserved between mouse and man: I) the extracellular domain; 2) the single cysteine; 3) the intramembrane aspartate; 4) all intracellular tyrosines; and 5) the possible ATP binding consensus sequence. As would be expected, in contrast to the high degree of conservation in the protein sequence of the mature subunit, the leader sequence is only 50% conserved.

ROLE OF ZETA IN THE EXPRESSION OF THE TCR

In studies of the biosynthesis, assembly and fate of the components of the TCR in murine antigen specific T cell hybridomas, we no ted that four of the chains of the TCR, alpha, beta, delta and epsilon were synthesized in great excess of the amount that survived to be expressed at the cell surface16• The excess proteins were (90%) transported from the site of synthesis in the ER through the Golgi where carbohydrate processing was completed only to be rapidly degraded, most Iikely by transport to lysosomes. Two other chains, CD3-gamma and zeta, were degraded to a much lesser extent. This was particularly true of zeta for which there was Iittle rapid degradation following synthesis. These data, and other information, suggested that gamma and zeta were limiting for the assembly of complete receptor complexes in these cells and that only complete complexes were capable of avoiding this intracellular Iysosomal degradation and be efficiently transported to and stabiy expressed on the cell surface. It was thus particularly interesting and particularly fortunate to be able to study the post-biosynthetic fate of the TCR in a variant of the 2B4 hybridoma which was isolated and cloned at the NIH by Jon Ashwell17. This variant, termed MA 5.8, made normal amounts of alpha, beta and CD3-gamma, -delta and -epsilon but made no detectable zeta chain by metabolic pulse labeling. Northern blot analysis of RNA derived from MA 5.8 failed to detect any zeta mRNA in these cells. Southern blotting with the murine zeta cDNA revealed identical restriction fragments in MA 5.8 and in the parent cell, 2B4. The assembly and carbohydrate processing

of the TCR chains in these cells occurred with identical kinetics and to an identical extent to that seen in the parental cells. 22

References

1. Marrack, P. & Kappler, J. (1986) Adv. Immunol. 38, 1–24.PubMedCrossRefGoogle Scholar
2. Weiss, A., Imboden, J., Hardy, K., Manger, B., Terhorst, C. & Stobo, J. (1986) Ann. Rev. Immunol. 4, 593.Google Scholar
3. Samelson, L. E., Harford, J. B. & Klausner, R. D. (1985) Cell 43, 223–231.PubMedCrossRefGoogle Scholar
4. Oettgen, H. C., Pettey, C. L., Maloy, W. L. & Terhorst, C. (1986) Nature (London) 320, 272–275.CrossRefGoogle Scholar
5. Weissman, A. M., Samelson, L. E. & Klausner, R. D. (1986) Nature 324, 480–482.PubMedCrossRefGoogle Scholar
6. Weissman, A. M., Baniyash, M., Hou, D., Samelson, L. E., Burgess, W. H. & Klausner, R. D. (1988) Science, in press.Google Scholar
7. Kyte, J. & Doolittle, R. (1982) J. Mol. Biol. 157, 105.Google Scholar
8. von Heijne, G. (1986) Nucl. Acids Res. 14, 4683.CrossRefGoogle Scholar
9. Van den Elsen, P., Shepley, B. A., Borst, J., Coligan, J. E., Markham, A. F., Orkin, S. & Cox, C. (1984) Nature 312, 413–418.PubMedCrossRefGoogle Scholar
10. Gold, D. P., Puck, J. M., Pettey, C. L., Cho, M., Coligan, J., Woody, J. N. & Terhorst, C. (1986) Nature 321, 431–434.PubMedCrossRefGoogle Scholar
11. Krissansen, G. W., Owen, M. J., Verbi, W. & Crumpton, M. J. (1986) EMBO J. 5, 1799–1808.PubMedGoogle Scholar
12. Saito, H., Kranz, D. M., Takagaki, Y., Hayday, A. C., Eisen, H. N. & Tonegawa, S. (1984) Nature 309, 757–762.PubMedCrossRefGoogle Scholar
13. Chien, Y-H., Iwashima, M., Kaplan, K. B., Elliot, J. F. & Davis, M. M. (1987) Nature 327, 677–682.PubMedCrossRefGoogle Scholar
14. Brenner, M. B., Dialynas, D. P., Strominger, J. L., Smith, J. A., Owen, F. L., Seidman, J. G., Ip, S., Rosen, F. & Krangel, M. S. (1986) Nature 302, 145–149.CrossRefGoogle Scholar
15. Kampo, M. P. et al. (1984) Nature 310, 589.CrossRefGoogle Scholar
16. Minami, Y., Weissman, A. M., Samelson, L. E. & Klausner, R. D. (1987) Proc. Natl. Acad. Sci. USA 84, 2688.Google Scholar
17. Sussman, J. J., Baonifacino, J. S., Lippincott-Schwartz, J., Sato, T., Klausner, R. D. & Ashwell, J. (1988) Cell 52, 85.PubMedCrossRefGoogle Scholar
18. Baniyash, M., Garcia-Morales, P., Bonifacino, J., Samelson, L. E. & Klausner, R. D. (1988) J. Biol. Chem., 263, 9874–9878.Google Scholar
19. Patel, M. D., Samelson, L. E. & Klausner, R. D. (1987) J. Biol. Chem. 262, 5831–5838.Google Scholar