4 Materials

4.1 Apparatus

Electroporation: Gene Pulser II	*BioRad*
Flow cytometry: FACS-Calibur	*Becton Dickinson*
Phospho-Imager: FLA-2000	*Fujifilm*
Microinjection moving table and microscope	*Olympus*
Microinjection pressure unit	*Eppendorf*
PCR thermocycler	*Applied Biosystems*
Protein electrophoresis and Western blotting	*NOVEX*
Protein chromatography: BioCAD SPRINT	*Perseptive Biosystems*

A list of suppliers with addresses and contact information is attached in the appendix.

4.2 Software

Tables, calculations, and statistics	GraphPad Prism 3.0 (GraphPad Software) and Excel 97 (Microsoft)
Graphics	Powerpoint 97 (Microsoft) and Photoshop 6.0 (Adobe Systems)
Flow cytometric analysis	Cell Quest 3.0 (BD) and FCS Express 1.0 (De Novo Software).
DNA sequence analysis	Clone manager 5.0 (Scientific & Educational Software)
Densitometry	Image Gauge 1.0 (FujiFilm)
Text	Word 97 (Microsoft)

Web resources:

DNA sequence identification: http://www.ncbi.nlm.nih.gov/BLAST/	Blast
DNA sequence comparison: http://www.ncbi.nlm.nih.gov/gorf/bl2.html	Blast2sequences

Oligonucleotide-primer design: Primer3
http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi

4.3 Chemicals, enzymes and kits

Protease inhibitors aprotenin, leupeptin, pepstatin A, phenylmethysulphonylflouride (PMSF), PEFA-block, propidium iodide, L-methionine, L-cysteine, chloroquine, and dextrane were obtained from *Sigma.* Zeocin was purchased from *Invitrogene.* Streptavidin and AP-conjugated streptavidin were obtained from *Roche*, PE-conjugated streptavidin from *Molecular Probes.* Deoxynucleotidetriphoshates were from *Gibco BRL.* If not otherwise stated, standard laboratory chemicals were purchased from *Sigma, Merck* or *Roth* in per analysi quality.
Radiochemicals: ^{32}P labeled α-dCTP and ^{51}Cr were from *Amersham*, and ^{35}S labeled methionine and cysteine (Tran-^{35}S-Label) were from *ICN*.
Synthetic peptides SALQNAASIA, amino acids 499-508 from mycobacterial hsp60 and KDIGNIISDA, amino acids 162-171 from murine hsp60 were supplied by Dr. Henklein, SFB 421.
Restriction enzymes were obtained from *New England Biolabs* . T4 ligase, reverse transcriptase, DNAse 1, RNAse H were purchased from *Gibco BRL*. Taq polymerase was purchased from *Gene Craft*, Pfu polymerase from *Stratagene*, Biotin protein ligase from *Avidity*.
The “Mini” and “Maxi“ plasmid purification kits, as well as the “QiaQuick” DNA gel extraction kit were obtained from *Qiagen*. The “Renaissance” Enhanced chemiluminescence kit was from *NEN.*

4.4 Organisms

Escherichia coli strain DH5α was used for standard procedures except for the expression of modified forms of H-2Kd and human β2m where *E. coli* strain BL-21 was used. Epicurian coli SURE® competent cells (*Stratagene*) were employed for the electroporation of large plasmids.
Mycobacterium bovis BCG (Bacillus de Calmette et Guérin) (Calmette and Guérin, 1909), strain Copenhagen, also known as BCG Danish was used for infections.

4.5 Mammalian cell lines

54ζ17, a TCR negative thymoma cell line overexpressing CD3ζ (Blank et al., 1993) was a kind gift from H.U. Weltzien, Freiburg. 54ζ17 cells were used as targets for retroviral transduction with constructs containing TCR chain cDNA.

58α⁻β⁻, another BW derived TCR negative thymoma cell line. 58α⁻β⁻ cells were used as targets for electroporation to test TCR expression vectors.

EL 4, derived from a lymphoma induced in a C57BL mouse. EL4 cells contain H2-b MHC class I molecules and served as target cells in ^{51}Cr release assays.

Phoenix ampho, a 293T cell based amphotrophic producer cell line containing stable integrations of *en* and *gag-pol* genes were kindly supplied by Garry Nolan, Stanford (Nolan, 2002).

4.6 Mice

All mice were bred and maintained under specific pathogen-free conditions in the animal facilities of the Max-Planck-Institute for Infection Biology at the Bundesamt für gesundheitlichen Verbraucherschutz und Veterinärmedizin (BgVV) according to the German animal protection law.

C57BL/6 mice, CD1 mice, and CBA mice were bred and supplied from the BgVV.

TCR α chain deficient mice on C57BL/6 genetic background were originally purchased from *The Jackson Laboratories*.

4.7 Media and Buffers

RPMI and DMEM media were obtained from *Biochrom*. Cell culture media were supplemented with 10% FCS, 0.2mM L-glutamine, 10 units/ml penicillin and streptomycin.

PBS: phosphate buffer saline (8 g NaCl, 0.2 g KCl, 0.2 g KH_2PO_4, 1.3 g Na_2HPO_4, ad 1000 ml) was used for washing steps in cell preparations and for FACS analysis.

4.8 Antibodies

An overview of the monoclonal antibodies (mAb) used is given in **Table 1.** mAb marked with an asterix (*) were purified from hybridoma supernatants by protein-G

sepharose. The mAb used for FACS analysis were conjugated with the fluorescent dyes FITC, PE or Cy-5. The mAb marked with a number sign (#) were used as isotype controls. IP = immunoprecipitation, CTL = cytolytic T lymphocyte ^{51}Cr release assay.

Table 1.

Specificity	Clone	Application	Source
TCR α chain constant region	H28-710	Western blot, IP	*Pharmingen*
TCR β chain constant region	H57-597	FACS, IP	*Pharmingen*
TCR Vα 8 segments	B21.14	FACS, CTL blocking	Gift from B. Malissen*
TCR Vβ 8.1, 8.2 segments	MR5-2	FACS, CTL blocking	*Pharmingen*
TCR γδ	GL3	Histology	ATCC*
CD4	YTS191.1	FACS	ATCC*
CD4	GK1.5	Histology	*Pharmingen*
CD3	145-2C11	FACS	*Pharmingen*
CD8a		FACS	ATCC*
CD44	IM7	FACS	*Pharmingen*
CD62L	MEL-14	FACS	*Pharmingen*
CD69	H1.2F3	FACS	*Pharmingen*
NK1.1	PK136 #	CTL blocking	ATCC*
Keyhole limpet hemocyanin Armenian hamster IgG2, λ	Ha4/8 #	IP	*Pharmingen*
Keyhole limpet hemocyanin Armenian hamster IgG2, κ	B81-3 #	IP	*Pharmingen*

4.9 Plasmids

Table 2 lists and describes the original plasmids used for cloning of the TCR genes. Plasmid charts are provided in the appendix.

Table 2.

Plasmid name	Description
pMSCV2.2-IRES-GFP	carries murine stem cell virus (MSCV) LTR containing coding sequence of green fluorescent protein (GFP) after an internal ribosomal entry sequence (IRES).
pP14αAR	contains full length P14 TCR α chain cDNA in pDPL13 plasmid
pP142β8AR	contains full length P14 TCR β chain cDNA in pDPL13 plasmid
pHSE3'	TCR cDNA expression vector containing H2-k promoter, β-globin splice site and poly-A signal, IgH enhancer (Pircher et al., 1989), pP14αAR, pP142β8AR, and pHSE3' were generously supplied by H.P. Pircher, Freiburg
pSHVα	TCR genomic expression vector derived from B10.A hybridoma, containing leader and 4 TCR Cα exons plus an IgH enhancer in the JC intron (Sakaguchi et al., 1994), pSHVα was a kind gift from Olivier Lantz, Villejuif.
pCR2.1	TA-cloning and sequencing vector, *Invitrogen*
pCMV/Zeo	contains Zeocin resistance under control of human CMV promoter, *Invitrogen*

4.10 Oligonucleotides

Table 3 gives a list of the synthetic oligonucleotides (PCR primers) used for cloning (C) of TCR vectors and screening (S) of genomic or cDNA from cell lines and transgenic mice. Standard primers were chosen using the web based software Primer3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). Oligonucleotides were obtained from *metabion*. The asterix (*) in the Vbeta8-Xho1-Ban-FW primer marks a silent mutation of the glycine codon at position 2 from GGC to GGG to avoid partial digestion with BanII.

Table 3.

Primer	**5'-3' sequence**	**application**
Alpha 7.2 FW	AGGGCGAAAAACTCACACTG	S
Alpha 7.2 REV	TGAAATGACAAGGCTGATGG	S
Alpha 8 FW	GAGGAGCAATGGAGATGGAG	S
Alpha 8 REV	GGACTTCTGCAGATGGAAGG	S
Beta 8.1 FW	AGCAAGGTGGCAGTAACAGG	S
Beta 8.1 REV	TCTCCTTTCTCCGTGCTGTC	S
Spec. alpha-construct	TTGCTTTCCTCTTTCCAAGC	S of pSHVα 7.2
Valpha8-Eco RI-FW	TCA**GAATTC**TTCTATGAACATGCGTCC	C + S of TCR α8, adds **EcoRI** site
Calpha-250-REV	GGAACGTCTGAACTGGGGTA	C + S of TCR α8
Calpha-912-REV	ATCCGGCTACTTTCAGCAGCAG	C + S of TCR α8
Vbeta8-Xho1-Ban-FW	TCACGT**CTCGAG**ATGGG**G***TCCAGACTCTTCT	C + S of TCR β8, adds **Xho1** site
Cbeta-762-REV	GCCTCTGCACTGATGTTCTG	C + S of TCR β8
Cbeta-Freer-REV	CTGTGTGACAGGTTTGGGTGA	C + S of TCR β8
UZ-α-7.2 (FW)	AAA**CTCGAG**ACCTGTGTGGATAAAAACCTCTCTGATTCTGGTTTGTCTTTTCTGTTTCCAAGC**AG**|TTGTGGCCCAGAAAGTGATTCAG	C of pSHVα 7.2, adds **Xho1** site, and **splice**|**acceptor**

Primer	**5'-3' sequence**	**application**
UZ-α-7.2R (REV)	AAAATT**GCGGCCGC**TTTGGCCCAAG AAACTGTCATCAAAACGT|**AC**TGGG CTTGATAGATAACTT	C of pSHVα 7.2, adds **NotI** site, and **splice|donor**
Neo-323-fw	CTCCTGCCGAGAAAGTATCCA	S of TCRα$^{-/-}$ mice
Neo-628-rev	CACAGTCGATGAATCCAGAAAAG	S of TCRα$^{-/-}$ mice
b-actin-fw	TGGAATCCTGTGGCATCCATGAAAC	RT-PCR +control
b-actin-rev	TAAAACGCAGCTCAGTAACAGTCCG	RT-PCR +control