ELSEVIER

Toxicology 107 (1996) 141–151

TOXICOLOGY

IMMUNOTOXICOLOGY

Review article

T cell immune responses to haptens. Structural models for allergic and autoimmune reactions

Hans Ulrich Weltzien*, Corinne Moulon, Stefan Martin, Elisabetta Padovan, Ute Hartmann, Jochen Kohler

Max-Planck-Institut für Immunbiologie, Stübeweg 51, D-79108 Freiburg, Germany

Received 30 May 1995; accepted 11 September 1995

Abstract

Protein-reactive chemicals, metal salts and drugs, commonly classified as immunological haptens, are major environmental noxes targeted at the immune system of vertebrates. They may not only interfere with this defense system by toxicity alone, but more often by evoking hapten-specific immune responses resulting in allergic and eventually autoimmune responses. Here, we review recent developements in the analysis of the structural basis of hapten recognition, particularly by T lymphocytes, which represent central elements in cell-mediated, as well as in IgE-dependent, allergies. A break-through in this field was the finding that T cells detect haptens as structural entities, attached covalently or by complexation to self-peptides anchored in binding grooves of major histocompatibility antigens (MHC-proteins). Synthetic hapten-peptide conjugates were shown to induce hapten-specific contact sensitivity in mice, opening new routes for studying hapten-induced immune disorders.

Keywords: TNP; Nickel; Penicillin; MHC; Peptide; Recognition; Receptor

1. Introduction

T lymphocytes comprise a central part of the immune defense system in vertebrates. Their clonally distributed antigen receptors (TCR) are specialized to interact with antigens exclusively on cellular surfaces in conjunction with class I ($CD8^+$ cytotoxic T cells) or class II ($CD4^+$ helper T cells) gene products of the major histocompatibility gene complex (MHC) (Davis and Chien, 1993; von Boehmer, 1994). This phenomenon is known as MHC-restricted antigen recognition (Zinkernagel and Doherty, 1974) and results from stringent selection processes during T cell maturation in the thymus (von Boehmer, 1994). Structurally, it relates to the tight association of 'processed' antigenic peptides with allele-specific peptide-binding grooves in the MHC molecules. It is this complex

Abbreviations: CS, contact sensitivity; CTL, cytotoxic T lymphocyte; LC, Langerhans cell; MHC, major histocompatibility complex; TNBS, trinitrobenzene sulfonic acid; TCR, T cell receptor; TNCB, trinitrochlorobenzene; TNP, trinitrophenyl.

* Corresponding author.

0300-483X/96/$15.00
SSDI 0300-483X(95)03253-C

surface of MHC plus peptide which contacts the TCR and eventually 'activates' the respective T cell population. Typically, T cells thus are focussed on the detection of protein determinants.

In addition, it has long been known that T cells may also react to a multitude of chemical reagents, metal salts and drugs, so-called haptens, in an antigen-specific and MHC-restricted way (Pohlit et al., 1979). Typical examples of protein-modifying haptens are chemicals such as trinitrochlorobenzene (TNCB) or trinitrobenzene sulfonic acid (TNBS) (Shearer, 1974), drugs like penicillins (Koponen et al., 1986), metal ions (Kapsenberg et al., 1987; Romagnoli et al., 1992; Sinigaglia, 1994) or natural compounds like urushiol in poison ivy (Kalish et al., 1994). Such reagents, besides their academic interest, raised particular attention as potent inducers of allergic and in some cases also autoimmune diseases (De Weck, 1990). They require binding to carrier proteins to become antigenic and it has been discussed whether or not T cells may require covalent modification of MHC molecules for hapten recognition. Recent results from several laboratories have pointed to a major role of hapten-modified, MHC-associated peptides as T cell-antigenic structures (Luescher et al., 1992; Nalefski and Rao, 1993; Martin and Weltzien, 1994; Sinigaglia, 1994; Cavani et al., 1995; Kohler et al., 1995), opening the way for detailed studies of hapten-T cell interactions by use of synthetic hapten-peptide conjugates.

2. Hapten determinants for T cells

Despite several lines of evidence indicating that haptens do not necessarily need to be linked covalently to MHC structures to be recognized by T cells (Schmitt-Verhulst et al., 1978; Levy and Shearer, 1982), it was only in 1992 that Ortmann et al. (1992) first activated T cells with synthetic, MHC-specific hapten-peptide conjugates. So far all structurally defined hapten-peptide epitopes belong to the group of model haptens such as tri- or dinitrophenyl groups (TNP or DNP) (Martin et al., 1993b; Kohler et al., 1995), azobenzene arsonate (Nalefski and Rao, 1993), photoreactive azido compounds (Romero et al., 1993; Luescher et al., 1994), etc., and little is known about structures of relevance for hapten-induced human immune disorders. Most of these model systems clearly proved that the hapten is, indeed, part of the antigenic structure. However, it should not be forgotten that hapten allergens may also function indirectly by altering the intracellular processing of self proteins (Griem and Gleichmann, 1994; Kubicka-Muranyi et al., 1994).

2.1. MHC class I restricted determinants

The knowledge on peptide-MHC interactions is exceedingly more detailed for class I than class II MHC molecules. Thus, crystallographic analyses are available for several human and mouse class I molecules complexed to defined peptides (Bjorkman et al., 1987; Jardetzky et al., 1991; Fremont et al., 1992, 1995), whereas such information is limited so far to one human class II structure (Brown et al., 1993). Secondly, MHC allele-specific peptide anchoring motifs are much better defined for class I than class II molecules (Rammensee, 1995). It is for these reasons that we and others have started the search for hapten-peptide epitopes with class I MHC-restricted T cells. Our own contribution to this system concentrated exclusively on H-2K^b-restricted, TNP-specific cytotoxic T cell (CTL) clones or CD8-transfected hybridomas thereof. K^b-binding peptides typically contain eight amino acids with Phe or Tyr in position 5 and Leu or other hydrophobic aliphatic amino acids in position 8 as anchors (Rammensee, 1995). Tyr in position 3 may serve as an additional anchor (Fremont et al., 1992; Matsumura et al., 1992; Shibata et al., 1992). Since these studies have been reviewed previously (Weltzien, 1992; Martin and Weltzien, 1994), we will summarize here only the main conclusions.

First, TNP-specific CTL induced by stimulation with antigen presenting cells that were modified chemically with trinitrobenzene sulfonic acid (TNBS, specific for Lys ϵ-amino groups) in general reacted to K^b-associating TNP-peptides and not to covalently TNP-modified K^b (von Bonin et al., 1992, 1993). We cannot exclude the existence of T cells of the latter specificity, but their frequency must be low. Secondly, whenever we identified an antigenic TNP-peptide for TNBS-induced CTL, it

carried the TNP-modification in position 4 of the octameric sequence (Martin et al., 1992). Thirdly, many of the TNP_4-specific CTL crossreactively reacted to TNP in position 4 of several different peptides, i.e. in a largely carrier-independent fashion (Martin et al., 1992).

This resulted in a model of an immunodominant TNP-determinant for K^b-restricted CTL as depicted schematically in Fig. 1A: TCR make contact predominantly to the hapten TNP and parts of the K^b molecule. The carrier peptide in this case serves mainly to anchor and position the hapten on the MHC-surface. For a notable proportion of TNP-specific CTL, chemical modification of intact cells with TNBS, therefore, results in a much higher density of crossreactive TNP-epitopes than is to be expected for any nominal peptide antigen. The highly repetitive pattern of determinants probably allows for primary activation even of low affinity T cells, and may explain the high frequency of T cells responding to this hapten (Hamann et al., 1983; Iglesias et al., 1992). It also may be responsible for the allergenic properties of TNCB.

The use of synthetic TNP-peptides further allowed for specific in vitro activation of CTL by presentation of only one defined type of determinant. Again, we found that T cells reacting to position 4-modified peptides were most easily triggered (Martin et al., 1993b). However, we also induced T cells specific for hapten-peptides carrying TNP-Lys in the peripheral position 7 of their sequences (Martin et al., 1993b). These CTL differed remarkably from the above mentioned species in that all of them, in addition to their K^b-restricted reactivity to TNP, exhibited recognition specificities for the carrier peptides. In almost every case, this peptide-reactivity was strong enough to also allow for lysis of target cells pulsed with the homologous, unmodified peptides. In fact, we identified two sub-epitopes for these CTL: one being the hapten TNP, the other a determinant formed by the side chains of amino acids 3 and 4 in the carrier peptides (see Fig. 1B). We have recently discussed the possibility that in case of self peptides, hapten modification might lead to the triggering of T cells which, once activated, might also react to the unmodified self structures, resulting in autoimmune phenomena (Martin et al., 1993b; Martin and Weltzien, 1994).

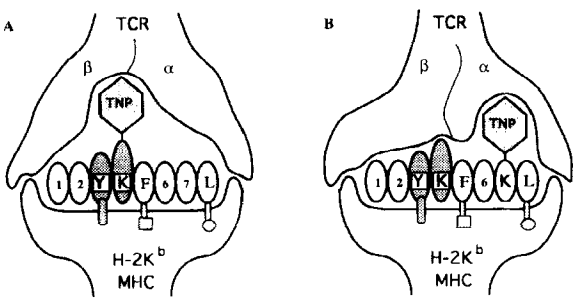

Fig. 1. Two types of H-2K^b-restricted TNP determinants. The immuno-dominant TNP-determinant on H-2K^b contains the hapten bound to lysine in the central position 4 of K^b-associated octapeptides (A). Many receptors recognizing this determinant predominantly contact the hapten itself in addition to haplotype-specific MHC-structures, but barely interact with side chains of the carrier peptide. In contrast, T cells specific for peripherally (position 7) modified TNP-peptides (B), contact the hapten-peptide via two independent sub-epitopes: one represented by TNP, the other by amino acids in carrier positions 3 and 4. Fig. 1A, B also depict the major anchoring amino acids F and L in positions 5 and 8 as well as the optional anchor Y in position 3.

2.2. MHC class II restricted determinants

Despite reports indicating that $CD8^+$ T cells may be involved in allergic contact sensitivity (CS) reactions (Gocinski and Tigelaar, 1990), a major role in CS is generally attributed to the Th1 subpopulation of the $CD4^+$ T cell compartment (Askenase, 1992). We have, therefore, initiated studies to structurally define major TNP-epitopes for class II MHC-restricted T cells in two strains of mice, i.e. C57BL/6 (H-2^b) and BALB/c (H-2^d). We found that roughly 25 or 50% of TNBS-reactive hybridomas or Th-lines, respectively, crossreactively recognized syngeneic presenter cells treated with tryptic digests of TNP-modified bovine serum albumin (TNP-BSA) or chicken egg albumin (TNP-OVA) or both (Kohler et al., 1995). Although there exists no simple way to study directly the role of covalently TNP-modified mouse class II molecules, the high degree of crossreaction of TNBS-induced Th cells with only two arbitrarily chosen TNP-proteins pointed to a major role of TNP-peptides as hapten-

determinants also for CD4+ T cells. This point was stressed by the finding, that lysine-containing I-A^b or I-A^d binding peptides of various origins (Staphylococcus Nuclease 93-105 (Finnegan et al., 1986), mouse IgGVH 59-69 (Rudensky et al., 1992), pigeon cytochrome *c* 45-58 (Suzuki and Schwarz, 1986) and λ-repressor 12-26 (Guillet et al., 1986)) when modified with TNP, constituted crossreactive determinants for a number of TNBS-specific T cell clones and hybridomas of appropriate restriction specificities. These reactions were inhibited by anti class II or anti TNP antibodies, demonstrating MHC-restricted recognition as well as a participation of the hapten itself in the antigenic epitope (Kohler et al., 1995).

Similar experiments were carried out by Cavani et al. (1995) using synthetic TNP-peptides binding to I-A^k (hen egg lysozyme 52-61) or I-A^u (polyA-based designer peptide). Again, hapten-specific crossreactivities with TNP-reactive helper cells induced on TNBS-modified stimulators (with no prior contact to the carrier peptides) were easily identified. On the basis of bulk cultures the authors identified immunodominant positions of TNP in these peptides.

In a different type of experiment (J.K., Doctoral Thesis, University of Freiburg, 1995), we induced I-A^b-restricted, TNP-specific T cells by in vivo immunization with several synthetic TNP-peptides. The majority of the resulting T cell lines and the T cell hybridomas obtained upon their fusion with a TCR-less thymoma again crossreacted with TNBS-modified stimulators. Furthermore, several carefully cloned hybridomas, i.e. individual TCR, were found to crossreact with TNP on various different peptides (see Table 1 for a particularly crossreactive hybridoma). We are, therefore, convinced that CD4+ as CD8+ T cells recognize TNP mainly in the form of MHC-associated, haptenated peptides, and that the immunodominant TNP-epitopes are largely independent of the carriers amino acid sequence.

2.3. TNP-peptides prime for contact sensitivity

Cell modification with TNBS, thus, produces carrier independent TNP-determinants for class I as well as for class II MHC-restricted T cells. Also CD4+ T helper cells may, therefore, encounter highly repetitive patterns of hapten epitopes on TNBS-treated presenter cells. Hence, the arguments discussed in Section 2.1 concerning the possible effects of repetitive determinants on the outcome of individual immune responses may also apply for CD4+ T cells. In this regard, it is interesting to note that we found many but by far not all TNP-specific Th cells to secrete the Th1-specific cytokine IFNγ (Table 2). Depending on the inducing antigen, between 5 and 14% of I-A^b/TNP-specific T cell lines secreted IL-4 but no

Table 1
Carrier-independent recognition of TNP by hybridoma H6A11.B3

Modification of stimulating cells[a]		IL-2 secretion (counts/min)[b]
Control		6.600 ± 310
TNBS		48.000 ± 1.390
TNP-peptides[c]	Peptide sequence	
1 IgGVH-TNP	GGNADFK* TPATLT	42.500 ± 1.640
2 Sendai-TNP	QPMLFK* TSIPKLA	53.000 ± 1.620
3 BSA (381)-TNP	EEAAAK* DDPHAAYS	45.000 ± 2.940
4 BSA (564)-TNP	EEQLK* TVMENFVA	45.000 ± 6.030
TNP-proteins[d]		
TNP-BSA		35.000 ± 2.000
TNP-OVA		21.000 ± 880
TNP-KLH		35.000 ± 1.990

[a]X-irradiated (3000 rad) spleen cells (C57BL/6) were either unmodified (control) or pretreated with 3 mM TNBS (trinitrobenzene sulfonic acid), 25 μg/ml of the peptides (peptide No 4 at 100 μg/ml), or 500 μg/ml of the TNP-proteins. Four times 10^5 stimulators were co-cultured for 24 h with 1×10^5 hybridoma cells in 200 μl supplemented Iscove's medium.

[b]Aliquots of culture supernatants were assayed for promotion of proliferation ([^{3}H]thymidine incorporation) on IL-2 dependent CTLL cells. Data show mean of triplicates with standard deviations.

[c]Synthetic, I-A^b-binding peptides containing TNP-lysine (K*). Sequences were derived from: (1) mouse IgG heavy chain, pos. 59-69 (Rudensky et al., 1992), extended by 2 Gly residues; (2) Sendai virus nucleoprotein 559-570 (Cole et al., 1994); (3) bovine serum albumin (BSA) 381-394 with Cys in positions 383, 384 and 392 replaced by Ala; (4) BSA 564-576.

[d]Proteins modified with 10 mM TNBS were BSA, chicken egg albumin (OVA) and Keyhole limpet hemocyanine (KLH). No responses were observed with the respective unmodified peptides or proteins (data not shown).

Table 2
Secretion of IFNγ and IL-4 by TNP-specific, I-A^b-restricted T cell lines

Inducing antigen	Sequence	Total number of lines	Secretors of		
			IFNγ	IL-4	IL-4 + IFNγ
TNBS		6	4	0	2
IgGVH-TNP[a]	GGNADFK*TPATLT	66	24	9	33
PCC-TNP[b]	GFSYTDANK*NKGIT	15	12	1	2
Nase-TNP[c]	YADGK*MVNEALVR	10	3	1	6

C57BL/6 mice were immunized either by skin painting with TNCB or s.c. with peptides in incomplete Freund's adjuvant. CD4$^+$ T cell lines were established from draining lymph nodes in each case in 96 individual microcultures. After six restimulations on irradiated syngeneic spleen cells modified either with 3 mM TNBS or the respective peptides (10 μg/ml), aliquots of the culture supernatants were assayed for IFNγ and IL-4 by Elisa. Shown are the total numbers of growing cultures and those producing significant amounts of either one or both interleukins. Note that the cultures were not cloned. Peptides contained TNP-Lys at indicated positions (K*).

Their sequences originate from:
[a]Mouse IgG (Rudensky et al., 1992) (see Table 1).
[b]Pigeon cytochrome *c* 45-58 (Suzuki and Schwarz, 1986).
[c]Staphylococcal nuclease 93-105 (Finnegan et al., 1986).

IFNγ and 10–60% produced both interleukins (Table 2). Th1 cells are implicated by most investigators as an essential component in the induction of CS (Askenase, 1992).

Consequently, we found that mice of H-2^b (Kohler et al., 1995) or H-2^d (Fig. 2) haplotypes may be sensitized by subcutaneous injection of class II MHC-binding TNP-peptides in incomplete Freund's adjuvant for the elicitation of CS (earswelling) with picrylchloride (TNCB) (Kohler et al., 1995). As shown in Fig. 2, the peptide-induced reactions were weaker as compared to priming with TNCB, but the results were repeated in several independent experiments (Kohler et al., 1995). In addition, Fig. 2 clearly demonstrates hapten-specificity: no earswelling upon challenge with oxazolone, and no induction of CS with the unmodified peptide λ-Rep. It should be stressed here that the CS reactions were elicited by application of pure TNCB to the ear so that only mouse proteins or peptides could be modified. The crossreaction with TNP on synthetic peptides of microbial origin such as staphylococcal nuclease (Kohler et al., 1995) or the λ-repressor (Fig. 2), therefore, strongly indicates a carrier independent reaction also in vivo. Moreover, the induction of CS with selectively class II MHC-specific TNP-

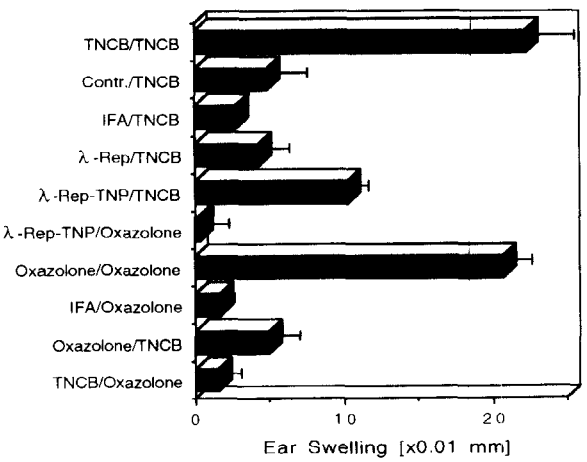

Fig. 2. Induction of contact sensitivity by synthetic TNP-peptides. BALB/c mice were either unimmunized (Contr.), sham-immunized with incomplete Freund's adjuvant (IFA), skin-painted with TNCB (7% in acetone) or oxazolone (3% in ethanol), or injected s.c. with 100 μg of peptide in IFA. After 5 days, one ear was treated with either 1% TNCB in acetone or 1% oxazolone in ethanol and ear swelling determined 24 h later. The peptide λ-Rep was derived from the λ-repressor 12-26 (LEDARRLK*AIYEKKK), known to bind to I-A^d (Guillet et al., 1986), and was used with and without TNP-modification on Lys-19. Data show mean of triplicates with standard deviations. Similar data have recently been published for C57BL/6 mice and I-A^b-binding TNP-peptides (Kohler et al., 1995).

peptides questions the necessity of $CD8^+$ T cells in this reaction. It is interesting in this context that no earswelling reaction was obtained with TNCB in C57BL/6 mice which had been injected with several different K^b-specific peptides carrying TNP in position 4 or 7 (J.K., Doctoral Thesis, University of Freiburg, 1995). It remains to be shown whether this failure to prime for CS is due to a lack of in vivo activation of $CD8^+$ T cells by class I MHC-binding TNP-peptides, to a lack of induction of CS by class I MHC-restricted T cells, or to a lack of IL-2 as a consequence of the missing activation of $CD4^+$ T cells.

2.4. Generation of hapten determinants on presenter cells

Model haptens such as TNCB or TNBS, but also clinically relevant reagents like penicillins or metal salts, interact with proteins in an indiscriminate way. Particularly reagents such as TNCB, TNBS or lactam-antibiotics which covalently link to lysine ε-amino groups are expected to modify any protein in their vicinity. The situation for metal ions such as Ni^{2+} may be somewhat more restrictive since sterically defined positioning of several amino acid ligands is required to form stable coordination-complexes. Still, even under these conditions a multitude of proteins will be modified, and MHC molecules will not be excluded.

In addition, most of the haptenic reagents are not restricted to react with soluble or cell surface proteins. They may eventually diffuse through membranes and modify cytoplasmic proteins. In that respect, it is of interest that TNP-conjugated ovalbumin has been introduced into the cytosol via acid-labile liposomes. There it was processed like the unmodified protein, and the major epitopes, i.e. the two octapeptides SIINFEKL and $SIINFEK_{TNP}L$, both were transported into the ER and presented on K^b molecules to the respective specific CTL clones (Martin et al., 1993a).

For class II restricted hapten-specific T cells, in vitro and in vivo stimulation with haptenated proteins were standard procedures in various laboratories for many years. It was assumed that hapten-proteins like other antigens may be endocytosed, processed and peptides re-exposed on the cellular surface in association with class II MHC molecules.

However, for class I (Ortmann et al., 1992) as well as class II molecules (Fig. 3) we demonstrated by haptenization of fixed cells that MHC-associated peptides may also be directly modified on the surface of living cells. As shown in Fig. 3, glutaraldehyde fixation of BALB/c splenocytes did not prevent their potential to present TNP to the hapten-specific hybridoma IG2-9 when modified chemically with TNBS or with the synthetic peptide λ-Rep-TNP. On the other hand, the presentation of TNP-ovalbumin was reduced to control levels by the fixation procedure, revealing successful blocking of the intracellular processing pathways. These findings are in agreement with reports of Nalefski et al. (1993) and Kalish et al.

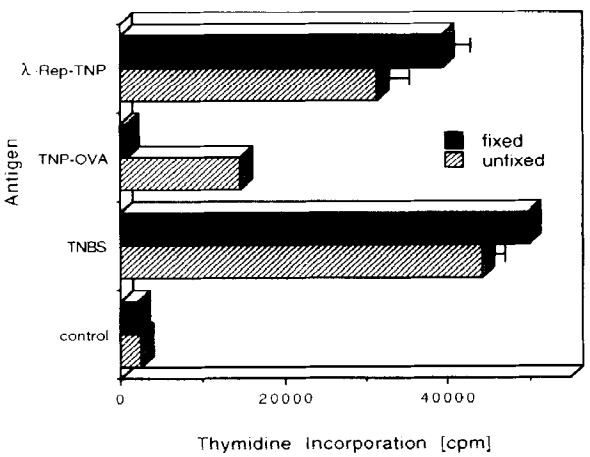

Fig. 3. TNBS-produced TNP-determinants on cell surfaces do not require intracellular processing. The I-A^d-restricted, TNP-specific T cell hybridoma IG2-9 derived from a BALB/c mouse was stimulated for 24 h on unfixed or glutaraldehyde fixed, irradiated BALB/c spleen cells. The stimulator cells were either unmodified or treated with 3 mM TNBS. Secreted IL-2 was determined by proliferation of an IL-2-dependent cell line as for data in Table 1. Controls were carried out on unmodified stimulators without TNP or in the presence of either 25 μg/ml of the peptide λ-Rep-TNP (see Fig. 2) or 1 mg/ml of the protein TNP-OVA (see Table 1). TNP-OVA, which requires intracellular processing, was recognized only on unfixed stimulators, whereas λ-Rep-TNP, just as TNBS-modified stimulators, activated independent of fixation. Data show mean of triplicates with standard deviations for T cell proliferation as determined by [^{3}H]thymidine incorporation.

(1994) concerning the MHC-restricted presentation of azobenzenearsonate and urushiol, respectively. Ma et al. (1994), in contrast, found a necessity of antigen processing for TNBS-modified BALB/c stimulators. This latter finding could not be reproduced under our conditions.

In general, thus, the generation of antigenic determinants during skin sensitization with reactive haptens may be the result of at least four different mechanisms: (1) modification of soluble proteins which may be endocytosed by Langerhans cells (LC) and presented as hapten-peptides on MHC class II after intracellular processing; (2) a similar route may be envisaged for modified membrane-proteins (non-MHC) of LC; (3) direct binding of the hapten to peptides already associated with class II (or class I) MHC molecules on the LC surface; (4) finally, the reactive chemicals might penetrate the plasma membrane and modify cytoplasmic proteins which then will be processed and presented preferentially on class I MHC. The latter mechanism would be particularly applicable for 'pro-haptens' requiring intracellular metabolism to be converted into protein-reactive molecules. The question whether and to what extent covalently haptenized MHC class II molecules may also represent T cell antigenic epitopes can not be answered conclusively.

2.5. *Hapten-reactive human T cells*

As mentioned above, the structural identification of hapten determinants of relevance in chemically induced human allergies is still at an early stage. There is no reason, however, to doubt that these T cell reactions follow principally similar routes as those defined in the mouse. Thus, Moulon et al. (1993) have shown that human T cells can be activated for primary in vitro responses to TNP just as in the mouse system, and that TNBS-modified human LC are a highly potent stimulator population. Sinigaglia et al. (1985) and Kapsenberg et al. (1987) demonstrated that from skin lesions or peripheral blood of nickel-allergic patients Ni-specific T cells could be cultivated which proliferated specifically on MHC class II matching presenter cells in the presence of Ni salts. Moreover, Romagnoli et al. (1991) showed that Ni salts inhibited unrelated peptide-specific T cell reactions if the antigenic peptide contained histidine, known as a typical Ni-complexing amino acid. It is still not known, however, whether such Ni-peptide-complexes, indeed, form the determinants recognized by Ni-specific T cells or whether Ni, for example, interferes with the processing of self-proteins to create immunogenic neo-determinants as postulated for the action of gold and mercury by Gleichmann and co-workers (Griem and Gleichmann, 1994; Kubicka-Muranyi et al., 1994). As a further step in identifying Ni-determinants, we have recently produced Ni-specific human T cell clones, many of which do not require the permanent presence of an excess of $NiSO_4$, but proliferate on stimulators only pulsed with Ni. Some reactivities of a typical representative of these clones (clone 11) are shown in the lower part of Fig. 4, demonstrating that these cells

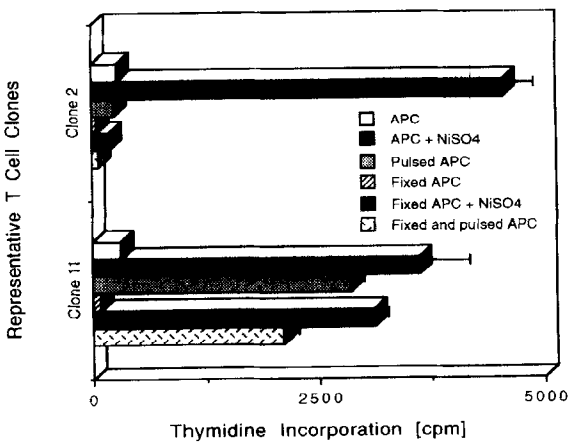

Fig. 4. Reactivity of representative Ni-specific human T cell clones. Peripheral blood cells of a Ni-sensitive individual were activated with 10^{-4} M $NiSO_4$ and cloned on allogeneic feeders in the presence of phythohaemagglutinin and IL-2. T cell clones were $CD4^+$ and MHC class II restricted. Antigen presenting cells (APC) in specificity assays were autologous, EBV-transformed B cells. $NiSO_4$ was either permanently present in the cultures (APC + $NiSO_4$) or APC were washed after 1.5 h incubation with 10^{-4} M $NiSO_4$ at 37°C and then used as presenters (pulsed APC). Fixation of APC was done in 0.05% glutaraldehyde for 45 s at room temperature. The reaction was stopped by addition of 0.2 M L-lysine and the cells were washed prior to the addition of $NiSO_4$. Data show mean of triplicates with standard deviations for T cell proliferation as determined by [^{3}H]thymidine incorporation.

were even triggered by fixed, Ni-pulsed presenter cells. This experiment for the first time directly points to the Ni^{2+} ion as participating in a determinant involved in T cell activation. It remains to be shown, however, whether this determinant is generated by complex formation of Ni with an MHC-associated peptide or with the MHC-molecule itself or whether MHC and peptide both contribute coordination bonds.

Other less frequent clones, represented by clone 2 in the upper part of Fig. 4, exhibit a different behaviour: they only react to presenter cells in the constant presence of $NiSO_4$. Neither pulsed nor fixed stimulators support their proliferation. In these cases, an effect of Ni on the processing of self proteins and the promotion of cryptic self-determinants has to be taken into consideration.

Interesting information has been provided by the group of Pichler on human T cells with specificity for penicillins and other drugs (Bell and Pichler, 1989; Brander et al. 1995; Mauri-Hellweg et al, 1995). Peripheral T cells of individuals allergic to various drugs were shown to specifically proliferate to autologous or MHC-matching stimulators in the presence of the appropriate drugs. Both, $CD4^+$ and $CD8^+$ T cells of such specificities were described, and for sulfamethoxazole-specific cells a bias for Vβ17-expressing antigen receptors was observed in one patient (Mauri-Hellweg et al., 1995). Moreover, also in this system, fixed cells pulsed with penicillin were shown to function as antigen presenters, provided they expressed the relevant restriction molecules (Brander et al., 1995). Reactivities of human T cells were also reported to penicillin-modified proteins, although these cells were not in all cases found crossreactive with penicillin-modified cells (Brander et al., 1995). Taken together, these data again point to the hapten penicillin as representing at least part of the relevant antigenic determinants for T cells, possibly in the form of modified, MHC-associated peptides. The latter statement, however, remains to be experimentally proven.

Some penicillin-reactive T cells exhibit exquisite structural specificity for particular penicillin-derivatives. Thus, we found that five of 11 penicillin G-induced T cell clones derived from one individual donor reacted to penicillin G but not to penicillin V or ampicillin, one recognized penicillins G and V, and five were stimulated by all three antibiotics (see Fig. 5 for typical examples of the three reactivity patterns). The first two types of reactivity clearly correlate to structural differences in the side chains of the penicillins. It remains to be shown whether type III T cells reflect a specificity for the common heterocyclic part of penicillins as discussed in a recent review by Blanca et al. (1994) or rather a broad crossreactive interaction with different phenylated side chains.

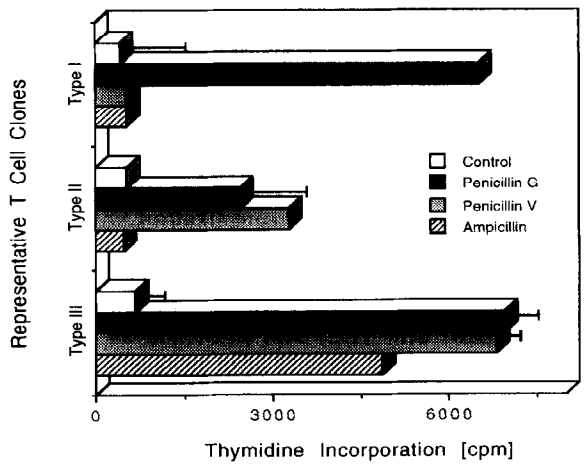

Fig. 5. Representative specificity patterns of penicillin G-induced human T cell clones. Peripheral blood cells of a patient with a history of hypersensitivity to penicillin G were stimulated with the same antibiotic and subsequently cloned on allogeneic feeders in the presence of phythohaemagglutinin and IL-2. Clones were assayed for antigen-specific proliferation ([^{3}H]thymidine incorporation) on autologous, EBV-transformed B cells in the presence of either penicillin G (side chain C_6H_5—CH_2—CO), penicillin V (C_6H_5—O—CH_2—CO) or ampicillin (C_6H_5—$CH(NH_2)$—CO). The core structure of all 3 β-lactam antibiotics is identical. Out of 11 clones isolated, five were of type I, five of type III and one of type II. Data show mean of triplicates with standard deviations for T cell proliferation as determined by [^{3}H]thymidine incorporation.

3. Concluding remarks

An important aspect of modern toxicology deals with interactions of chemicals and drugs with the immune system. Besides typical toxic effects such

reagents or haptens may also interfere in a more specific way with the hosts defense system. Thus, they often lead to hapten-specific cellular and humoral hyper-responsiveness, manifested in B and/or T cell-mediated allergic reactions. The structural basis for hapten-specific T cell reactions is particularly poorly understood. Recent work on model haptens in our and others laboratories has opened this field for molecularly orientated research. In this brief review, we have tried to cover this progress and hopefully succeeded to demonstrate that the field is only just beginning to escape from the descriptional phase.

One of the most important findings is that haptens when conjugated to MHC-associated peptides form, indeed, structural parts of the epitopes contacting the antigen-specific receptors of T cells. Moreover, haptenated peptides bound to MHC appear to represent the dominant part of the hapten-determinants recognized by $CD8^+$ as well as by $CD4^+$ T cells. This finding opened the way to use modern peptide synthesis to study T cell-hapten interactions in great detail. Two types of recognition of such hapten-peptides by T cells were defined: one independent and one dependent of the carrier peptide sequence. We put forward hypotheses implying that these two ways of hapten recognition may relate to hapten-induced allergic or autoimmune responses, respectively. Regarding chemically induced allergies, we showed that synthetic, class II MHC-binding TNP-peptides were capable of inducing a contact sensitivity reaction apparently without participation of TNP-specific $CD8^+$ T cells. The experimental system of synthetic hapten-conjugated peptides, thus, harbours great potential for future in vitro as well as in vivo analyses of hapten-specific immune responses.

Acknowledgements

Work in the authors laboratory was funded in part by the Deutsche Forschungsgemeinschaft and the Federal Ministry for Bildung, Wissenschaft, Forschung und Technologie. J.K. was recipient of a fellowship of the Evangelisches Studienwerk e.V.; S.M. received the Otto Hahn medal and fellowship of the Max-Planck-Society.

Note added in proof

The following relevant articles appeared or were accepted for publication during processing of this manuscript:

Martin, S., Ruh, H., Hebbelmann, S., Pflugfelder, U., Rüde, B., Weltzien, H.U. (1995) Carrier-reactive hapten-specific cytotoxic T lymphocyte clones originate from a highly preselected T cell repertoire: implications for chemical-induced self-reactivity. Eur. J. Immunol. 25, 2788–2796.

Moulon, C., Vollmer, J., Weltzien, H.U. (1995) Characterization of processing requirements and metal cross-reactivities in T cell clones from patients with allergic contact dermatitis to nickel. Eur. J. Immunol. 25, in press.

Padovan, E., Mauri-Hellweg, D., Pichler, W.J., Weltzien, H.U. (1995/6) T cell recognition of penicillin G: structural features determining antigenic specificity. Eur. J. Immunol., in press.

Fernandez, M., Warbrick, E.V., Blanca, M., Coleman, J.W. (1995) Activation and hapten inhibition of mast cells sensitized with monoclonal IGE anti-penicillin antibodies: evidence for two-site recognition of the penicillin-derived determinant. Eur. J. Immunol. 25, 2486–2491.

References

Askenase, P.W. (1992) Delayed-type hypersensitivity recruitment of T-cell subsets via antigen-specific non-IgE factors or IgE antibodies — relevance to asthma, autoimmunity and immune-responses to tumors and parasites. Chem. Immunol. 54, 166–211.

Bell, S.J.D. and Pichler, W.J. (1989) Penicillin-allergic patients react to penicillin-modified 'self'. Allergy 44, 199–203.

Bjorkman, P.J., Saper, M.A., Samaroui, B., Bennett, W.S., Strominger, J.L. and Wiley, D.C. (1987) The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. Nature 329, 512–518.

Blanca, M., Vega, J.M., Garcia, J., Miranda, A., Carmona, M.J., Juarez, C., Terrados, S. and Fernandez, J. (1994) New aspects of allergic reactions to betalactams: crossreactions and unique specificities. Clin. Exp. Allergy 24, 407–415.

Brander, C., Mauri-Hellweg, D., Bettens, F., Rolli, H., Goldman, M. and Pichler, W.J. (1995) Heterogeneous T cell responses to β-lactam-modified self-structures are observed in penicillin-allergic individuals. J. Immunol. (in press).

Brown, D., Jardetzky, T.S., Gorga, J.C., Stern, L.J., Urban, R.G., Strominger, J.L. and Wiley, D.C. (1993) Three-

dimensional structure of the human class II histocompatibility antigen, HLA-DR1. Nature 364, 33–39.

Cavani, A., Hackett, C.J., Wilson, K.J., Rothbard, J.B. and Katz, S.I. (1995) Characterization of epitopes recognized by hapten-specific CD4+ T-cells. J. Immunol. 154, 1232–1238.

Cole, G.A., Katz, J.M., Hogg, T.L., Ryan, K.W., Portner, A. and Woodland, D.L. (1994) Analysis of the primary T-cell response to Sendai virus-infection in C57BL/6 mice: CD4+ T-cell recognition is directed predominantly to the hemagglutinin-neuraminidase glycoprotein. J. Virol. 68, 6863–6870.

Davis, M.M. and Chien, Y.-H. (1993) Topology and affinity of T cell receptor mediated recognition of peptide-MHC complexes. Curr. Opin. Immunol. 5, 45–49.

De Weck, A.L. (1990) Drug allergy, immunotherapy, immune complexes and anaphylaxis. Curr. Opin. Immunol. 2, 548–557.

Finnegan, A., Smith, M., Smith, J., Berzofsky, J., Sachs, D. and Hodes, R. (1986) The T cell repertoire for recognition of a phylogenetically distant protein. J. Exp. Med. 164, 897–910.

Fremont, D.H., Matsumura, M., Stura, E.A., Peterson, P.A. and Wilson, I.A. (1992) Crystal structures of two viral peptides in complex with murine MHC class I H-2K^b. Science 257, 919–927.

Fremont, D.H., Stura, E.A., Matsumura, M., Peterson, P.A. and Wilson, I.A. (1995) Crystal structure of an H-2K^b-ovalbumin peptide complex reveals the interplay of primary and secondary anchor positions in the major histocompatibility complex binding groove. Proc. Natl. Acad. Sci. USA 92, 2479–2483.

Gocinski, B.L. and Tigelaar, R.E. (1990) Roles of CD4+ and CD8+ T cells in murine contact sensitivity revealed by in vivo monoclonal antibody depletion. J. Immunol. 144, 4121–4128.

Griem, P. and Gleichmann, E. (1994) Interaction of antigen with Au(III), a reactive metabolite of antirheumatic gold drugs, leads to sensitization of T cells to cryptic epitopes. Immunobiology 191, 224–224.

Guillet, J., Lai, M., Briner, T., Smith, J. and Gefter, M. (1986) Interaction of peptide antigens and class II major histocompatibility complex antigens. Nature 324, 260–262.

Hamann, U., Eichmann, K. and Krammer, P.H. (1983) Frequencies and regulation of trinitrophenyl-specific cytotoxic T precursor cells: Immunization results in release from suppression. J. Immunol. 130, 7–14.

Iglesias, A., Hansen-Hagge, T., Martin, S., von Bonin, A., Eichmann, K., Ortmann, B. and Weltzien, H.U. (1992) Increased frequency of 2,4,6-trinitrophenyl (TNP)-specific cytotoxic T lymphocyte precursors in transgenic mice expressing a T cell receptor β-chain gene from a H-2K^b-restricted, TNP-specific cytolytic T cell clone. Eur. J. Immunol. 22, 863–866.

Jardetzky, T.S., Lane, W.S., Robinson, R.A., Madden, D.R. and Wiley, D.C. (1991) Identification of self peptides bound to purified HLA-B27. Nature 353, 326–329.

Kalish, R.S., Wood, J.A. and LaPorte, A. (1994) Processing of urushiol (poison ivy) hapten by both endogenous and exogenous pathways for presentation to T cells in vitro. J. Clin. Invest. 93, 2039–2047.

Kapsenberg, M.L., Res, P., Bos, J.D., Schootemijer, A., Teunissen, M.B.M. and Schooten, W.V. (1987) Nickel-specific T lymphocyte clones derived from allergic nickel-contact dermatitis lesions in man: heterogeneity based on requirement of dendritic antigen-presenting cell subsets. Eur. J. Immunol. 17, 861–865.

Kohler, J., Martin, S., Pflugfelder, U., Ruh, H., Vollmer, J. and Weltzien, H.U. (1995) Cross-reactive trinitrophenylated peptides as antigens for class II major histocompatibility complex-restricted T cells and inducers of contact sensitivity in mice. Limited T cell receptor repertoire. Eur. J. Immunol. 25, 92–101.

Koponen, M., Pichler, W.J. and de Weck, A.L. (1986) T cell reactivity to penicillin: phenotypic analysis of in vitro activated cell subsets. J. Allergy Clin. Immunol. 78, 645–652.

Kubicka-Muranyi, M., Griem, P., Lübben, B., Lührmann, R. and Gleichmann, E. (1994) Mercuric chloride alters nuclear self antigen, upregulates its presentation, and activates specific T helper cells for auto-antibody formation. Immunobiology 191, 225–226.

Levy, R.B. and Shearer, G.M. (1982) Cell mediated lympholysis responses against autologous cells modified with haptenic sulfhydryl reagents. J. Immunol. 129, 1525–1529.

Luescher, I.F., Cerottini, J.C. and Romero, P. (1994) Photoaffinity-labeling of the T-cell receptor on cloned cytotoxic T-lymphocytes by covalent photoreactive ligand. J. Biol. Chem. 269, 5574–5582.

Luescher, I.F., Loez, J.A., Malissen, B. and Cerottini, J.C. (1992) Interaction of antigenic peptides with MHC class I molecules on living cells studied by photoaffinity labelling. J. Immunol. 148, 1003–1011.

Ma, J., Wang, J.H., Sy, M.S., Guo, Y.J., Hauser, C. and Bigby, M. (1994) Trinitrophenol reactive T-cell hybridomas recognize antigens that require antigen-processing. J. Invest. Dermatol. 103, 42–48.

Martin, S., Ortmann, B., Pflugfelder, U., Birsner, U. and Weltzien, H.U. (1992) Role of hapten-anchoring peptides in defining hapten-epitopes for MHC-restricted cytotoxic T-cells: cross-reactive TNP-determinants on different peptides. J. Immunol. 149, 2569–2575.

Martin, S., Niedermann, G., Leipner, C., Eichmann, K. and Weltzien, H.U. (1993a) Intracellular processing of hapten-modified protein for MHC class-I presentation: cytoplasmic delivery by pH-sensitive liposomes. Immunol. Lett. 37, 97–102.

Martin, S., von Bonin, A., Fessler, C., Pflugfelder, U. and Weltzien, H.U. (1993b) Structural complexity of antigenic determinants for class I MHC-restricted, hapten-specific T cells: two qualitatively differing types of H-2K^b-restricted TNP epitopes. J. Immunol. 151, 678–687.

Martin, S. and Weltzien, H.U. (1994) T cell recognition of haptens, a molecular view. Int. Arch. Allergy Immunol. 104, 10–16.

Matsumura, M., Fremont, D.H., Peterson, P.A. and Wilson,

I.A. (1992) Emerging principles for the recognition of peptide antigens by MHC class I molecules. Science 257, 927–964.

Mauri-Hellweg, D., Bettens, F., Mauri, D., Brander, C., Hunziker, T. and Pichler, W.J. (1995) Activation of drug-specific $CD4^+$ and $CD8^+$ T cells in individuals allergic to sulfonamides, phenytoin and carbamazepine. J. Immunol. 155, 462–472.

Moulon, C., Peguet-Navarro, J., Courtellemont, P., Redziniak, G. and Schmitt, D. (1993) In vitro primary sensitization and restimulation of hapten-specific T-cells by fresh and cultured human epidermal Langerhans cells. Immunology 80, 373–379.

Nalefski, E.A. and Rao, A. (1993) Nature of the ligand recognized by a hapten-specific and carrier-specific, MHC-restricted T-cell receptor. J. Immunol. 150, 3806–3816.

Ortmann, B., Martin, S., von Bonin, A., Schiltz, E., Hoschützky, H. and Weltzien, H.U. (1992) Synthetic peptides anchor T cell-specific TNP-epitopes to major histocompatibility antigens. J. Immunol. 148, 1445–1450.

Pohlit, H., Haas, W. and von Boehmer, H. (1979) Cytotoxic T cell responses to haptenated cells. I. Primary, secondary and long-term cultures. Eur. J. Immunol. 9, 681–690.

Rammensee, H.-G. (1995) MHC ligands and peptide motifs: first listing. Immunogenetics 41, 178–228.

Romagnoli, P., Labhardt, A.M. and Sinigaglia, F. (1991) Selective interaction of Ni with an MHC-bound peptide. EMBO J. 10, 1303–1306.

Romagnoli, P., Spinas, G.A. and Sinigaglia, F. (1992) Gold-specific T cells in rheumatoid arthritis patients treated with gold. J. Clin. Invest. 89, 254–258.

Romero, P., Casanova, J.L., Cerottini, J.C., Maryanski, J.L. and Luescher, I.F. (1993) Differential-T cell-receptor photoaffinity-labeling among $H\text{-}2K^d$ restricted cytotoxic T-lymphocyte clones specific for a photoreactive peptide derivative — labeling of the alpha-chain correlates with J-alpha segment usage. J. Exp. Med. 177, 1247–1256.

Rudensky, A.Y., Preston Hurlburt, P., Alramadi, B.K., Rothbard, J. and Janeway, C.A. (1992) Truncation variants of peptides isolated from MHC class-II molecules suggest sequence motifs. Nature 359, 429–431.

Schmitt-Verhulst, A.-M., Pettinelli, C.B., Henkart, P.A., Lunney, J.K. and Shearer, G.M. (1978) H-2-restricted cytotoxic effectors generated in vitro by the addition of trinitrophenyl-conjugated soluble proteins. J. Exp. Med. 174, 352–368.

Shearer, G.M. (1974) Cell-mediated cytotoxicity to trinitrophenyl-modified syngeneic lymphocytes. Eur. J. Immunol. 4, 527–533.

Shibata, K.-I., Imarai, M., van Bleek, G.M. and Nathenson, S.G. (1992) Vesicular stomatitis virus antigenic octapeptide N52-59 is anchored into the groove of the $H\text{-}2K^b$ molecule by the side chains of three amino acids and the main chain atoms of the amino terminus. Proc. Natl. Acad. Sci. USA 89, 3135–3139.

Sinigaglia, F. (1994) The molecular basis of metal recognition by T cells. J. Invest. Dermatol. 102, 398–401.

Sinigaglia, F., Scheidegger, D., Garotta, G., Scheper, R., Pletscher, M. and Lanzavecchia, A. (1985) Isolation and characterization of Ni-specific T cell clones from patients with Ni-contact dermatitis. J. Immunol. 135, 3929–3932.

Suzuki, G. and Schwarz, R. (1986) The pigeon cytochrome *c*-specific T cell response of low responder mice. J. Immunol. 136, 230–239.

von Boehmer, H. (1994) Positive selection of lymphocytes. Cell 76, 219–228.

von Bonin, A., Ortmann, B., Martin, S. and Weltzien, H.U. (1992) Peptide-conjugated hapten groups are the major antigenic determinants for trinitrophenyl-specific cytotoxic T cells. Int. Immunol. 4, 869–874.

von Bonin, A., Martin, S., Plaga, S., Hebbelmann, S. and Weltzien, H.U. (1993) Purified MHC class I molecules present hapten-conjugated peptides to $TNP/H\text{-}2K^b$-specific T cell hybridomas. Immunol. Lett. 35, 63–68.

Weltzien, H.U. (1992) Renaissance for hapten-specific T lymphocytes: implications for basic and applied research. Z. Naturforsch. 47c, 323–328.

Zinkernagel, R.M. and Doherty, P.C. (1974) Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis with a syngeneic or semiallogeneic system. Nature 248, 701–702.