

Short communication

Allergy to antibiotics: T-cell recognition of amoxicillin is HLA-DR restricted and does not require antigen processing

Horton H, Weston SD, Hewitt CRA. Allergy to antibiotics: T-cell recognition of amoxicillin is HLA-DR restricted and does not require antigen processing.

Allergy 1998; 53: 83–88. © Munksgaard 1998.

Allergic immune responses are initiated and maintained by T cells that recognize peptidic fragments of allergens in the context of major histocompatibility complex (MHC) class II molecules. An anomaly of this model exists in the T-cell response to haptens. Haptens are nonpeptide antigens that alone are too small to provoke an immune response. Nevertheless, T-cell responses to haptenic allergens clearly occur and are critically involved in allergic immune responses to drugs such as penicillin. Although the mechanisms that generate T-cell epitopes from protein antigens are well understood, haptens create T-cell epitopes by alternative mechanisms. These may include binding of haptens directly to preformed MHC-peptide complexes on the cell surface, or indirect association with MHC molecules after conjugation with self cell surface or serum proteins that are then processed and presented as haptenated peptide antigens. Which of these unorthodox mechanisms of epitope generation is dominant in allergy to penicillin is unknown. This study aims to determine the nature of the epitopes recognized by amoxicillin-specific T cells from allergic donors, and to clarify whether T-cell responses to penicillin antibiotics are MHC-restricted and require haptenated self proteins to be processed before recognition. Human T-cell lines specific for amoxicillin were raised and used in assays with processing-disabled and MHC-class II-typed antigen-presenting cells to determine the MHC restriction and processing requirements of T cells recognizing amoxicillin. Fixation of antigen-presenting cells with paraformaldehyde, before or after pulsing with amoxicillin, established that T cells can recognize amoxicillin-containing epitopes with a similar efficiency irrespective of whether the antigenic conjugate has been internalized and processed. These results suggest that amoxicillin can bind directly to preformed MHC-peptide complexes and need not necessarily involve the processing of haptenated self carrier proteins before recognition of the conjugate by amoxicillin-specific T cells.

Antibiotics are among the most frequent causes of allergic drug reactions. It is estimated that up to 10% of patients treated with β -lactam antibiotics experience some form of immunologic hypersensitivity to the drug (1). Truly allergic, IgE-mediated reactions to penicillins range from mild urticaria to life-threatening anaphylaxis (2–4). Non-IgE-mediated, but immunologic reactions to penicillin also occur and include haemolytic anaemia and vascu-

litis or nephritis caused by antipenicillin antibodies interacting with penicillinated erythrocytes or soluble proteins, respectively (2, 6, 8). Dermatologic manifestations of hypersensitivity to penicillins are also common and include rashes, dermatitis, and fixed drug eruptions (2, 9, 10).

The danger of provoking a serious allergic reaction to penicillin complicates the clinical management of patients with cystic fibrosis and bacterial

**H. Horton¹, S. D. Weston²,
C. R. A. Hewitt²**

¹Immunotoxicology Laboratory, Centre for the Mechanisms of Human Toxicity, Hodgkin Building, University of Leicester, Leicester;

²Transplantation Laboratory, Department of Surgery, Leicester General Hospital, Leicester, UK

Key words: adverse drug reactions; antigen presentation; antigen processing; MHC class II; penicillin allergy; T cells.

Dr Colin R. A. Hewitt
Immunotoxicology Laboratory
Centre for the Mechanisms of Human Toxicity
Hodgkin Building
University of Leicester
PO Box 138
Lancaster Road
Leicester LE1 9HN
UK

Accepted for publication 13 August 1997

endocarditis who require frequent or prophylactic treatment with these drugs (3, 4). Although several alternative, less allergenic drugs are available, penicillins are often the drug of choice for treatment of brain abscesses, bacterial meningitis, and gestational syphilis or neurosyphilis (6, 7).

Penicillins have molecular weights in the range of 350–500 Da. Antigens of this size are too small to be immunogenic and are therefore classified as haptens. In common with other haptenic allergens, penicillins probably acquire immunogenicity by spontaneous conjugation to self carrier proteins *in vivo*. Whether immunogenic penicillin is bound to self cell surface proteins or to serum proteins, however, is a subject of some controversy (11, 12). It is also unclear whether immunogenic neoepitopes consist of penicillin bound to MHC molecules, or to peptides associated with MHC molecules. In the latter case, two mechanisms are possible: penicillin may bind to cell surface or soluble proteins that are processed into penicillinated peptides and loaded onto MHC molecules; alternatively, penicillin may bind directly to the peptide of a preformed complex (13–20). These novel mechanisms for epitope generation have provoked controversy as to whether penicillin-self protein conjugates always require processing before recognition by hapten allergen-specific T cells (13–20).

In this study we have raised T-cell lines from patients allergic to amoxicillin to determine the nature of naturally conjugated, immunogenic amoxicillin self protein conjugates. Specifically, we have sought to clarify whether these clinically relevant antihapten responses are MHC-restricted and require penicillin-self protein conjugates to be processed before recognition by T cells.

Material and methods

Patients

Subjects with a documented history of penicillin hypersensitivity were recruited from the Asthma and Allergy Research Centre at the Leicester General Hospital in accordance with local ethical committee guidelines. Patient E.R. had a 24-year-long history of penicillin allergy, and patient K.W. had suffered an urticarial rash after oral administration of amoxicillin 2 years before recruitment to the study.

Antibiotics and antibodies

Penicillin G, methicillin, dicloxacillin, oxacillin, ampicillin, and nafcillin were purchased from Sigma-Aldrich Chemical Co. Ltd (Dorset, UK). Amoxicillin, flucloxacillin, and temocillin were generously donated by SmithKline Beecham Pharmaceuticals plc (West Sussex, UK). Azlocillin was the generous

gift of Bayer Pharmaceutical (Berkshire, UK). Stock solutions of all penicillins were prepared at 100 mgml⁻¹ in water and stored at -20°C.

Culture media

Lymphocyte proliferation assays and T-cell lines were cultured in RPMI 1640 (Life Technologies, Inc, Paisley, UK) supplemented with 5% pooled, heat-inactivated human A⁺ serum (National Blood Transfusion Service, Sheffield, UK) and 2 mM L-glutamine (ARPMI). Epstein-Barr virus (EBV)-transformed B-cell lines were cultured in RPMI 1640 supplemented with 10% foetal calf serum (Sigma-Aldrich) and 2 mM L-glutamine (FRPMI). All cells were cultured without antibiotics.

Amoxicillin-specific T-cell lines

Peripheral blood mononuclear cells (PBMC) were prepared by density gradient centrifugation over LymphoprepTM (Nycomed, Oslo, Norway). PBMC were washed and cultured at 5×10⁶ cells well⁻¹ in the wells of a 24-well plate in ARPMI containing a final concentration of 1 mgml⁻¹ amoxicillin. Cultures were incubated at 37°C in a 5% CO₂, 100% humidity atmosphere. T-cell lines were maintained with 20 IUml⁻¹ recombinant human interleukin-2 (IL-2) (AldesleukinTM, Eurocetus, UK) every 2–3 days, and an equal number of 2500 R γ-irradiated syngeneic antigen-presenting cells (APC) and 1 mgml⁻¹ amoxicillin every 14 days.

Preparation of EBV-B-cell lines

EBV-transformed B-cell lines (EBV-B) were prepared by incubating 5×10⁶ freshly isolated PBMC with EBV-containing supernatant from the marmoset cell line B95.8 (gift of Dr Alan Rickinson, University of Birmingham, UK). After incubation for 1 h at 37°C, cells were resuspended in FRPMI containing 0.2 μgml⁻¹ cyclosporin A (Sandoz, Rugby, UK).

Proliferation assays

T cells (1×10⁴) were cocultured with 1×10⁴ 2500 R γ-irradiated syngeneic PBMC APC in a final volume of 200 μl in the wells of a 96-well, round-bottomed tissue culture plate. Each antibiotic was added at a range of concentrations from 0 to 2 mgml⁻¹. After 48 h of culture, each well was pulsed with 37 kBq [³H] thymidine (Amersham International plc, Amersham, UK) for 16 h. Cells were harvested onto glass fibre filters by a semi-automatic cell harvester, and the isotope incorporated was measured by scintillation spectroscopy (Wallac, Milton Keynes, UK). Results are shown

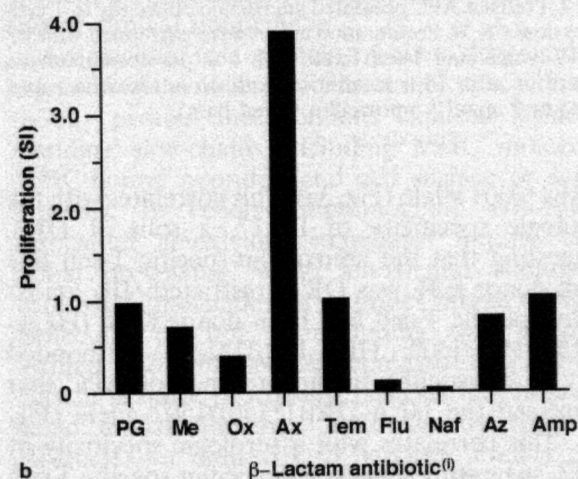
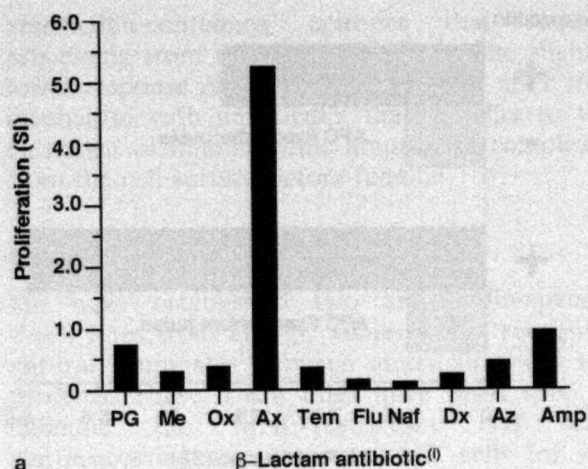


Fig. 1. Specificity of amoxicillin-reactive T-cell lines. a) T-cell lines from donor E.R. and b) donor K.W. were specific for amoxicillin. APC (10^4) were cocultured with equal number of T cells. Each antibiotic was used in proliferation assays at concentration of 1 mgml^{-1} . (i) PG: penicillin G; Me: methicillin; Ox: oxacillin; Ax: amoxicillin; Tem: temocillin; Flu: flucloxacillin; Naf: nafcillin; Dx: dicloxacillin; Az: azlocillin; Amp: ampicillin.

as the mean of triplicate cultures in counts per minute (cpm) or stimulation indices (SI). SI were calculated by dividing the mean cpm with antibiotic by the mean cpm without antibiotic. SI values greater than 2.5 were considered significant.

MHC class II typing of APC

EBV-B to be used as APC were typed for MHC class II genotype. Typing was carried out by polymerase chain reaction using HLA-DR β sequence-specific primers. Alleles for the DR β 1 loci were assigned with commercial primer kits (British Society for Histocompatibility and Immunogenetics, Molecular Special Interest Group, Bristol University, Bristol, UK).

Immunogenicity of amoxicillin

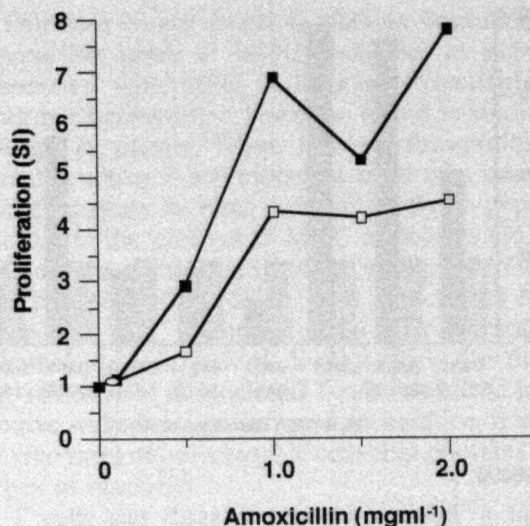


Fig. 2. Dose response of T-cell lines to amoxicillin. Amoxicillin-specific responses of T-cell lines from donors E.R. (filled squares) and K.W. (open squares) were dependent upon concentration of amoxicillin. Equal numbers of PBMC and T cells (10^4) were cocultured in proliferation assays with amoxicillin at $0\text{--}2 \text{ mgml}^{-1}$. Proliferation of each T-cell line was optimal at 1 mgml^{-1} .

MHC class II restriction of amoxicillin-specific T-cell lines

MHC class II restriction of the T-cell lines was confirmed with EBV-B APC prepared from MHC class II-typed donors expressing HLA-DR alleles matched or mismatched with those of the T-cell donors. Standard T-cell proliferation assays were conducted with 5000 R γ -irradiated EBV-B cells as APC in the presence and absence of an optimal concentration of amoxicillin.

Determination of antigen-processing requirements

EBV APC were pulsed for 16 h with 1 mgml^{-1} amoxicillin before or after fixation with paraformaldehyde. APC were fixed for 15 min with 1% paraformaldehyde (Sigma-Aldrich) in PBS at room temperature. Cells were washed once with PBS and incubated for 30 min in FRPMI at 37° , followed by two additional washes in PBS before use as APC in standard proliferation assays.

Results

T-cell lines

T-cell lines isolated from two amoxicillin-allergic subjects, E.R. and K.W., were confirmed to be specific for amoxicillin, as the cells failed to respond significantly to any other penicillin tested (Fig. 1a and b). The proliferative responses of both

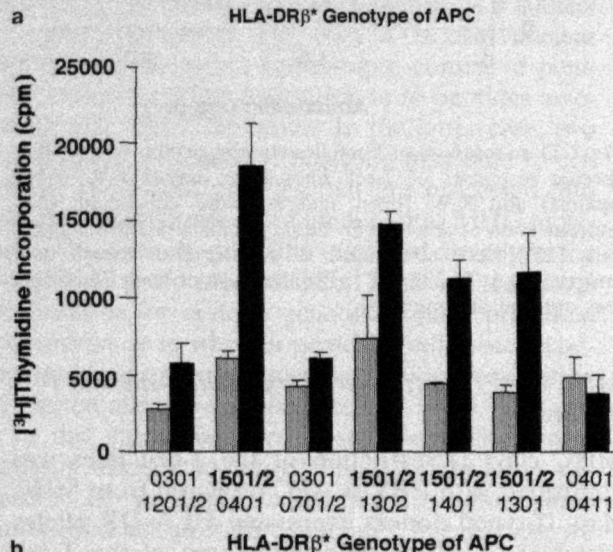
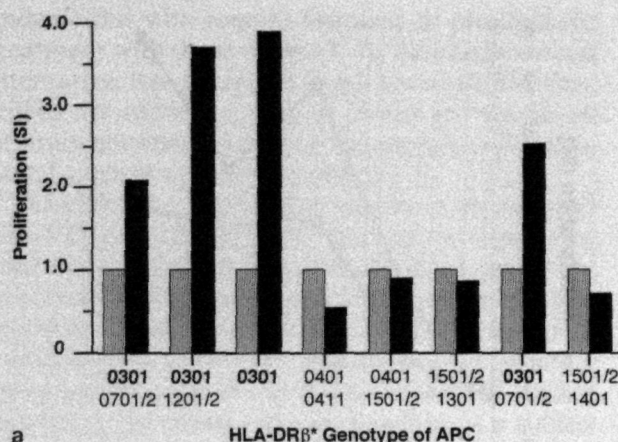


Fig. 3. MHC class II restriction of amoxicillin-specific T-cell lines. MHC class II restriction of a) E.R. and b) K.W. T-cell lines. Proliferation assays were set up with panel of different EBV-transformed B-cell lines matched or mismatched for HLA-DR alleles of E.R. or K.W. Gamma-irradiated EBV-B cells (10^4) were cultured with equal numbers of T cells and no amoxicillin (open bars), or 1 mg ml^{-1} amoxicillin (filled bars).

T-cell lines to amoxicillin was antigen dose-dependent, reaching a plateau between 1 and 2 mg ml^{-1} (Fig. 2).

MHC class II restriction elements of amoxicillin-specific T-cell lines

To determine whether the T-cell responses of the amoxicillin-specific T-cell lines were MHC class II restricted, a panel of MHC class II-typed EBV-B APC that shared some or none of the MHC class II alleles of E.R. and K.W. were used as APC in proliferation assays with the T-cell lines (Fig. 3a and b). The amoxicillin-specific T-cell line from donor E.R. (HLA-DRβ1*0301; HLA-DRβ1*0701/0702) responded only to amoxicillin in the presence of EBV-B cell APC that expressed the HLA-

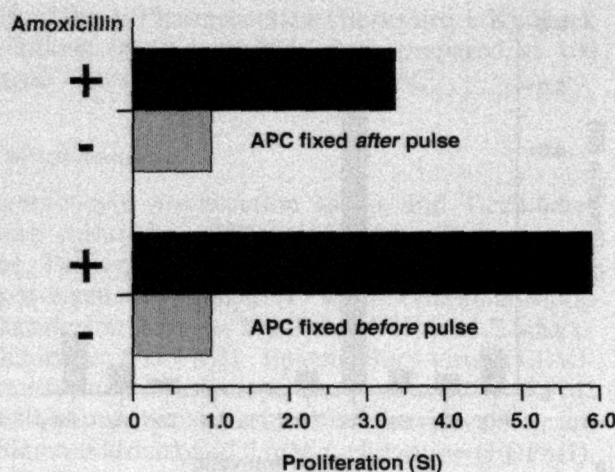


Fig. 4. Prefixed APC presented amoxicillin efficiently to T cells from donor K.W. Proliferation assays were performed with 10^4 APC, which had been fixed with 1% paraformaldehyde before or after 16-h incubation with no amoxicillin (open bars) or 1 mg ml^{-1} amoxicillin (filled bars).

DRβ1*0301 allele (Fig. 3a). This correlates with the serologic specificity of DR17, a split of DR3, suggesting that the amoxicillin-specific T-cell line from donor E.R. was DR17-restricted. The amoxicillin-specific T-cell line from donor K.W. (HLA-DRβ1*1501/1502; HLA-DRβ1*1302) responded only to amoxicillin in the presence of APC that expressed the HLA-DRβ1*1501/1502 allele (Fig. 3b). This correlates with a serologic specificity of DR2, suggesting that the amoxicillin-specific T-cell line from donor K.W. was DR2-restricted.

Processing requirements of naturally formed immunogenic amoxicillin conjugates

To determine whether naturally formed amoxicillin-self protein conjugates always require processing before recognition by amoxicillin-specific T cells, EBV-B APC were fixed with paraformaldehyde before or after pulsing with amoxicillin. APC fixed after a 16-h incubation with amoxicillin efficiently presented the drug to amoxicillin-specific T cells (Fig. 4). To determine whether these immunogenic complexes were formed after self carrier proteins had been processed and loaded as peptides onto MHC class II molecules, EBV-B APC were fixed before incubation with amoxicillin (Fig. 4). Under these conditions, EBV-B were slightly more efficient APC than those fixed after a 16-h incubation with the drug. This result strongly suggests that a substantial proportion of the response to amoxicillin is accounted for by direct conjugation of the drug with preformed MHC-peptide complexes that are already at the cell surface. The possibility remains, however, of a minor role for

amoxicillin-containing epitopes that require processing from native self proteins. The slightly lower response of T cells to APC fixed after 16-h incubation with amoxicillin may be due to the clearance of immunogenic amoxicillin complexes from the cell surface before fixation.

Discussion

We have established two amoxicillin-specific T-cell lines from allergic subjects that recognize natural conjugates between amoxicillin and self proteins. These T-cell lines have been used to examine the MHC-restriction and antigen-processing requirements of T cells for self protein-conjugated amoxicillin, an allergenic hapten of clinical importance.

Amoxicillin is a protein-reactive drug and, in common with other penicillin antibiotics, will bind to any protein that contains exposed lysine or histidine side-chains, including MHC molecules, MHC-bound peptides, and cell surface or serum proteins. To become immunogenic, non-MHC-associated amoxicillin conjugates require additional processing, while amoxicillin conjugated with MHC molecules, or peptides bound to MHC molecules do not. Our finding that naturally formed amoxicillin protein-conjugates do not necessarily require processing suggests that immunogenic amoxicillin may bind directly to MHC molecules, or indirectly to peptides already positioned in the grooves of MHC molecules. Our data argue against an alternative mechanism in which the drug binds to a native, non-MHC associated protein that is then processed to amoxicillin-containing, MHC-binding peptides.

The requirements of hapten-specific T cells for antigen processing are controversial. Some studies show that processing-disabled APC are unable to present haptens to T-cell hybridomas (21), while others conclude that processing is not always required for presentation of haptens to T-cell clones (19, 20). These discrepancies might be explained by the use of cloned T cells, where the unique and exquisite specificity of a clone may be a disadvantage. This is because the *in vitro* response of a T-cell clone measures only the recognition of a single epitope, the generation or expression of which may or may not be sensitive to inhibition by fixation. In contrast, oligoclonal T-cell lines may include clones of cells that recognize both processing-sensitive and -insensitive epitopes. This argument is supported by a report showing that processing was strictly required for the recognition of nickel by 40% of nickel-specific T-cell clones, while the remaining 60% of clones recognized nickel presented by fixed APC (20).

Our data do not establish whether immunogenic amoxicillin binds to MHC molecules or peptides associated with MHC molecules. Precedents for both mechanisms are, however, found in studies of allergy to metals. When antigen recognition by metal reactive T-cell clones is used as a readout, nickel appears to bind predominantly to peptides located in the grooves of MHC molecules (16, 17). This contrasts with the situation with gold, in which the hapten is thought to bind directly to MHC molecules (17). It has not been fully investigated whether these apparent discrepancies are due to the selection of monoclonal T-cell clones and hybridomas with these patterns of recognition from an *in vivo* pool of polyclonal T cells that contains both types of reactivity.

T cells can directly recognize either a hapten attached to *any* MHC-bound peptide (hapten-specific T cells), or a combination of parts from the hapten and peptide (conjugate-specific T cells) (22, 23). Since the frequency of each individual peptide epitope on the surface of an APC is very low, the surface density of epitopes recognized by conjugate-specific T cells will also be low. In contrast, haptens anchored to MHC molecules by *any* peptide are relatively frequent and present a high density of epitopes to hapten-specific T cells. This has led to the proposal of a mechanism, based on hapten recognition, by which immediate and delayed hypersensitivity may be induced by the same drug in different individuals (17). Anchoring of amoxicillin to MHC molecules by a large number of different peptides already within MHC molecules may promote a high ligand density for hapten-specific T-cell antigen receptors. High epitope densities on APC, such as those seen by hapten-specific T cells, are reported to favour the development of Th1 cells involved in type IV immunopathologies (17). Conversely, low-density epitopes, such as those recognized by conjugate-specific T cells, promote Th2 cell development and type I immunopathologies (17).

Acknowledgments

This work was funded by the Medical Research Council of Great Britain. We are grateful to Dr Martin Stern and Ms Elizabeth Emerson of the Asthma and Allergy Research Unit at Leicester Royal Infirmary for identifying penicillin-allergic patients and obtaining clinical samples.

References

1. Idsøe O, Guthe T, Willcox RR, de Weck AL. Nature and extent of penicillin side-reactions, with particular reference to fatalities from anaphylactic shock. *Bull World Health Organ* 1968;38:159-88.
2. de Weck AL. Penicillins and cephalosporins. In: *Allergic reactions to drugs. Handbook of experimental pharmacology*. Berlin: Springer, 1983:423-82.

3. Lin RY. A perspective on penicillin allergy. *Arch Intern Med* 1992;152:930-7.
4. Antibiotic treatment of bacterial endocarditis. *Drug Ther Bull* 1987;25:49-51.
5. Boguniewicz M, Leung DYM. Hypersensitivity reactions to antibiotics commonly used in children. *Pediatr Infect Dis J* 1995;14:221-31.
6. Weiss ME, Adkinson NF. Immediate hypersensitivity reactions to penicillin and related antibiotics. *Clin Allergy* 1988;18:515-40.
7. Wendel GD, Stark BJ, Janison RB, Molina RD, Sullivan TJ. Penicillin allergy and desensitisation in serious infections during pregnancy. *N Engl J Med* 1985;312:1229-32.
8. Werner M, Costa MJ, Mitchell LG, Nayar R. Nephrotoxicity of xenobiotics. *Clin Chim Acta* 1995;237:107-54.
9. Gamboa P, Jauregui I, Urrutia I, Gonzalez G, Antepara I. Contact sensitisation to cloxacillin with oral tolerance to other β -lactam antibiotics. *Contact Dermatitis* 1996;34:75-6.
10. Arias J, Fernandez-Rivas M, Panadero P. Selective fixed drug eruption to amoxycillin. *Clin Exp Dermatol* 1995;20:339-40.
11. Bell SJD, Pichler WJ. Penicillin-allergic patients react to penicillin-modified "self". *Allergy* 1989;44:199-203.
12. Lafaye P, Lapresle C. Fixation of penicilloyl groups to albumin and appearance of anti-penicilloyl antibodies in penicillin-treated patients. *J Clin Invest* 1988;82:7-12.
13. Martin S, Weltzien HU. T cell recognition of haptens, a molecular view. *Int Arch Allergy Immunol* 1994;104:10-16.
14. Claas FHJ, Ruina van Nieuwkoop R, van den Berge W, van Rood JJ. Interaction of penicillin with HLA-A and B antigens. *Hum Immunol* 1982;5:83-90.
15. Sinigaglia F, Scheidegger D, Garotta G, Scheper R, Pletscher M, Lanzavecchia A. Isolation and characterisation of Ni-specific T cell clones from patients with Ni-contact dermatitis. *J Immunol* 1985;135:3929-32.
16. Romagnoli P, Labhardt AM, Sinigaglia F. Selective interaction of Ni with an MHC-bound peptide. *EMBO J* 1991;10:1303-6.
17. Sinigaglia F. The molecular basis of metal recognition by T cells. *J Invest Dermatol* 1994;102:398-401.
18. Romagnoli P, Spinas GA, Sinigaglia F. Gold-specific T cells in rheumatoid arthritis patients treated with gold. *J Clin Invest* 1992;89:254-8.
19. Brander C, Mauri-Hellweg D, Bettens F, Rolli H, Goldman M, Pichler WJ. Heterogeneous T cell responses to β -lactam-modified self structures are observed in penicillin-allergic individuals. *J Immunol* 1995;155:2670-8.
20. Moulon C, Vollmer J, Weltzien HU. Characterisation of processing requirements and metal cross-reactivities in T cell clones from patients with contact dermatitis to nickel. *Eur J Immunol* 1995;25:3308-15.
21. Ma J, Wang J-H, Sy M-S, Guo Y-J, Hauser C, Bigby M. Trinitrophenol reactive T-cell hybridomas recognise antigens that require antigen processing. *J Invest Dermatol* 1994;103:42-8.
22. Martin S, von Bonin A, Fessler C, Pflugfelder U, Weltzien U. Structural complexity of antigenic determinants for class I MHC-restricted, hapten-specific T cells. *J Immunol* 1993;151:678-87.
23. Clayberger C, Dekruffy RH, Aisenberg J, Cantor H. Hapten-specific inducer T cells. I. Definition of two classes of hapten-specific inducer cells. *J Exp Med* 1983;157:1906-19.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.