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Structural requirements and biological significance of interactions between peptides and the major histocompatibility complex

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Previous studies indicate that T cells recognize a complex between the major histocompatibility complex (MHC) restriction-element and peptide-antigen fragments. Two aspects of this complex formation are considered in this paper: (1) what is the nature of the specificity of the interactions that allows a few MHC molecules to serve as restriction elements for a large universe of antigens; and (2) what is the relative contribution of determinant selection (i.e. antigen–MHC complex formation) and T-cell repertoire in determining the capacity of an individual to respond to an antigen? By analysing single amino acid substitution analogues of a peptide antigen (Ova 325–335) as well as by analysing the structural similarities between unrelated peptides capable of binding to the same MHC molecule, we have been able to document the very permissive nature of the antigen–MHC interaction. Despite this permissiveness of binding, it is possible to define certain structural features of peptides that are associated with the capacity to bind to a particular MHC specificity. With respect to the question of the relative role of 'determinant selection' and 'holes in the T-cell repertoire' in determining immune responsiveness, we present data that suggest both mechanisms operate in concert with one another. Thus only about 30% of a collection of peptides that in sum represent the sequence of a protein molecule were found to bind to Ia. Although immunogenicity was restricted to those peptides that were capable of binding to Ia (i.e. determinant selection was operative), we found that about 40% of Ia-binding peptides were not immunogenic (i.e. there were also 'holes in the T-cell repertoire').

1. Introduction

T cells recognize protein antigen in a complicated interaction with antigen-presenting cells (APCs). These cells do not recognize antigen directly, but only after the antigen has been 'processed' (physically altered by denaturation or fragmentation) by an APC (Shimonkevitz *et al.* 1983) and subsequently 'displayed' in association with major histocompatibility complex (MHC) molecules on the APC surface (Buus *et al.* 1987*b*; Allen *et al.* 1987). Previous studies on the mechanism of antigen recognition have established that T cells recognize a complex formed between MHC and peptides derived from protein antigens (Werdelin 1982; Watts *et al.* 1984; Buus *et al.* 1986), and that there is a strong correlation between the capacity to form complexes with peptides and the capacity to serve as the MHC restriction element used in the immune response to the same peptides (Babbitt *et al.* 1985; Buus *et al.* 1987*a*).

This paper considers two questions that arise from this concept of T-cell recognition of MHC–antigen complexes: (1) what is the nature of the specificity of the interaction between peptides and MHC that allows the few MHC molecules expressed by an individual capable of binding a large universe of antigenic peptides; and (2) what is the relative contribution of this determinant selection by MHC on defining the immune response to an antigen?

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2. Structural requirements for the interaction between Ia and antigen

To understand how a presumably large universe of immunogenic peptides can bind to and be restricted by the very small number of Ia specificities present within an individual, we have undertaken to characterize the requirements for the binding of peptides to a given MHC molecule. These studies were predominantly done by using the class II MHC restriction element IA^d and a set of peptides that were capable of binding to that MHC molecule. Much of the work has concentrated on the immunogenic peptide from chicken ovalbumin (Ova) 323–339. The first approach taken was to compare the structure of the binding region of Ova 323–339 with the binding regions of other unrelated peptides that were also capable of interacting with a high affinity with IA^d. To define the IA^d-interacting region within the peptide Ova 323–339, we synthesized a series of *N*- and *C*-terminal truncated analogues and tested these analogues for binding to purified IA^d molecules. By this procedure we were able to define a critical core region within Ova 323–339 that was involved in binding to IA^d. It consisted of the hexapeptide 327–332 and had the sequence Val-His-Ala-Ala-His-Ala.

We have identified six other peptides from unrelated proteins that also bind strongly to IA^d. As each of these peptides can competitively inhibit the binding of Ova 323–339 to IA^d, it is most likely that they all bind to the same site on IA^d as the Ova peptide, and therefore should share the critical structural features required for such binding. To estimate the structural relationship between these peptides, we adapted the methods described by Grantham (1974) and Padlan (1977) which compare, for any pair of amino acids, their structural dissimilarity, taking into account differences in atomic composition, polarity, and molecular volume. Using the core binding region of Ova as the 'master' sequence, we were able to identify in each of the other peptides the region that was most similar to this core IA^d binding region from the Ova peptide. These alignments are shown in table 1. The common structural motif that emerges from this set of alignments is: position 1 appears to be the most similar in the six peptides being occupied by the hydrophobic residues Val, Ile or Leu; the next position is occupied either by a basic residue (His, Arg or Lys) or the polar residue Thr; this is followed by two hydrophobic residues in positions 3 and 4; position 5 is more variable but tends to be occupied by polar or charged amino acids; finally, position 6 is occupied either by Ala or by two other residues with short or no sidechain, Ser or Gly. Although this motif could be discerned in the great majority of good IA^d-binding peptides, we have also identified IA^d-binding peptides that apparently do not share this motif. In a recent analysis of over 80 peptides, approximately three quarters of the strong binders to IA^d contained the above motif.

We have been able to confirm experimentally the involvement of these predicted core-binding regions in two of the peptides listed in table 1: influenza virus haemaglutinin (Ha) 130–142 and sperm whale myoglobin (Myo) 106–118. By doing similar types of *N*- and *C*-terminal truncation analysis as we had for Ova 323–339, we were able to define a core region within the Ha peptide that consists of the sequence Asn-Gly-Val-Thr-Ala-Ala-Cys-Ser as being critical for binding to IA^d. This sequence contains the predicted binding region based on the structural similarity analysis, Val-Thr-Ala-Ala-Cys-Ser. Similarly, for the myoglobin peptide, *N*- and *C*-terminal truncation analysis indicated that the region involved in IA^d binding was contained in the sequence Ala-Ile-Ile-His-Val-Leu-His-Ser. Again, the predicted region of similarity with the Ova peptide, Ile-His-Val-Leu-His-Ser, is contained within with IA^d-

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Table 1. Structural similarities between unrelated peptides with strong IA^d-binding capacity

peptide	residue number 1	2	3	4	5	6
Ova 327–332	Val	His	Ala	Ala	His	Ala
Ha 135–140	Val	Thr	Ala	Ala	Cys	Ser
Myo 66–71	Val	Thr	Val	Leu	Thr	Ala
Myo 112–117	Ile	His	Val	Leu	His	Ser
Nase 104–109	Val	Arg	Gln	Gly	Leu	Ala
Nase 15–20	Ile	Lys	Ala	Ile	Asp	Gly

binding region of the myoglobin peptide. Thus the structural similarities between these unrelated peptides that we have discerned on the basis of a pairwise comparison of the amino acids contained within these various peptides is indeed critical for the expression of IA^d-binding capacity by these peptides. A further indication that this is so is that we have been able to predict other peptides that can bind to IA^d on the basis of their possessing this motif, and have been able to experimentally verify this potential IA^d-binding capacity by synthesizing the peptide and confirming its IA^d-binding capacity.

To characterize further the relative contribution to IA^d binding of individual residues within the Ova 323–339 peptide, we synthesized a series of 55 single-substitution analogues of the peptide Ova 323–336, which is highly stimulatory for certain T cells and binds just as well as Ova 323–339 to IA^d. For each of the 11 positions, 325–335, we synthesized five analogues, each carrying a single amino acid substitution (two conservative, one semi-conservative and two non-conservative). When these analogues were tested for their capacity to bind to IA^d, it was found that most of the substitutions had little or no effect on Ia binding. Only nine out of 55 (16%) had a significant effect (reduction to less than one third), and only seven substitutions led to reduction to less than one fifth in IA^d-binding capacity (figure 1). The most dramatic changes were seen at residues Val 327 and Ala 332. These results are in agreement with those obtained with the series of truncated peptides and indicate that Val 327 and Ala 332 are the most critical residues involved in determining IA^d binding. Significant but less dramatic effects

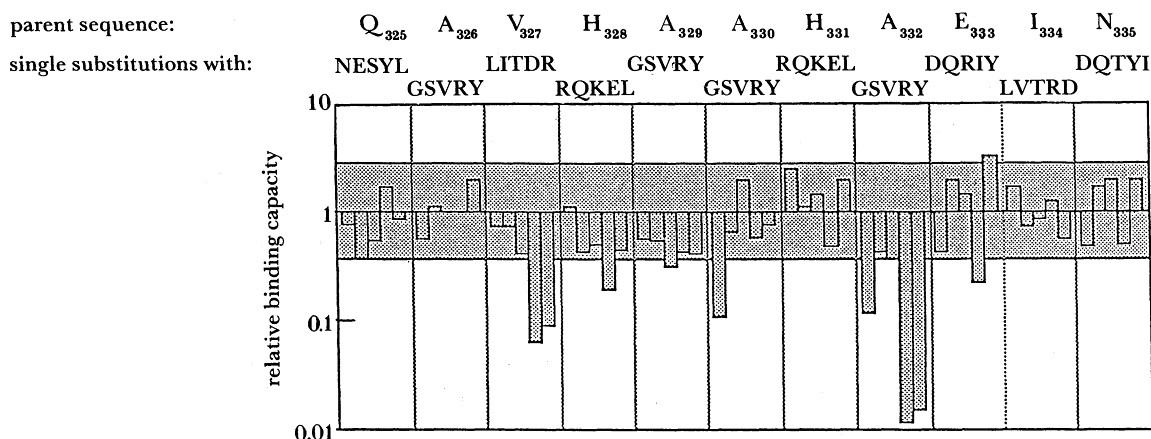

Figure 1. IA^d-interacting capacity of single-substitution analogues of Ova 323–336. Five different substitutions (two conservative, one semi-conservative, two non-conservative) were introduced into each of the eleven positions 323–335. These peptides were then tested for IA^d binding. Each bar represents the arithmetic mean of two to four independent determinations. The shaded area represents 99% confidence limits.

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were detected at His 328 and Glu 333. Although we have interpreted the decrease in IA^d-binding capacity associated with substitutions at these positions as being caused by alterations of IA^d contact residues, we cannot exclude the possibility that the decreased binding could be secondary to a change in peptide conformation caused by the substitutions.

In summary, these data confirm the very permissive nature of peptide binding to IA^d in that *ca.* 85% of the substitutions tested had little or no effect on the IA^d-binding capacity of Ova 323–336. These findings are in keeping with the determinant selection hypothesis and suggest the possible mechanism by which Ia molecules could bind many seemingly unrelated peptides.

3. The relative contribution of 'determinant selection' and 'holes in the T-cell repertoire' in defining T-cell responses to a protein antigen

Two main theories have been proposed to explain the MHC control of T-cell immune responses. According to one line of reasoning ('determinant selection'), MHC molecules act as specific antigen receptors, thereby allowing some, but not all, antigens to interact with a particular MHC and form potentially T-cell stimulatory moieties (Rosenthal 1978). The other line of reasoning ('holes in the T-cell repertoire') claims that MHC control of T-cell responses is at the level of the T cell itself and not at the level of the interaction between antigen and MHC (Klein 1982). In the broadest sense, such 'holes in the T cell repertoire' may be due to either an absence of the relevant T-cell receptor (caused by limitations of the T-cell receptor gene repertoire or by deletion of T cells) or, alternatively, by the unresponsiveness of antigen-specific T cells (caused by anergy or by suppression) (Paul 1984).

Because it is possible to study the formation of antigen–MHC complexes, we were in a position to evaluate the extent to which determinant selection influences the immune response to a foreign protein. For this study we have used a panel of 14 overlapping peptides representing the entire sequence of the protein staphylococcal nuclease (Nase) to estimate the frequency of Ia-binding sites within a protein antigen, and to evaluate the relative contribution of determinant selection as opposed to holes in the T-cell repertoire in the generation of T-cell responses to these peptides. Each peptide studied was 20 amino acids long and overlapped the sequence of the adjacent peptides by ten amino acids (Finnegan *et al.* 1986). This synthetic design was chosen to optimize the chances of identifying all T-cell determinants within the intact Nase. The size of these peptides is similar to the size of peptides that have been shown to bind to Ia and be immunogenic, and the overlapping nature of the peptides reduces the risk of missing a T-cell determinant owing to the arbitrarily chosen division between peptides.

The ability of this set of peptides to bind to the Ia molecules expressed by $H\text{-}2^d$ and $H\text{-}2^k$ mice was measured by their capacity to inhibit the binding of radiolabelled peptides that had been previously characterized for their capacity to bind to particular Ia specificities. Table 2 shows the capacity of these four different affinity purified Ia molecules to bind the panel of 14 Nase peptides. Five peptides, 21–40, 41–60, 51–70, 71–90 and 131–149, failed to bind any of the four Ia molecules tested, whereas one peptide, 101–120, bound to all four Ia specificities. The remaining peptides bound to one to three of the four Ia molecules. Given the sensitivity level of this assay (a 50% inhibition dose of 600 μM), five peptides were found to bind to IA^d, two to IE^d, three to IA^k and eight to IE^k. Owing to the overlapping nature of the peptides, the binding of two adjacent peptides to a particular MHC molecule may be caused by a single

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Table 2. Binding of a panel of 14 Nase peptides to four Ia molecules

(Dash indicates 50 % inhibition at over 600 μm.)

Nase peptide	concentration of Nase peptide needed to obtain 50 % inhibition of binding/μm			
	^{125}I-labelled Ova 323–339 to IA^d	^{125}I-labelled repr 12–26 to IE^d	^{125}I-labelled HEL 46–61 to IA^k	^{125}I-labelled Nase 101–120 to IE^k
Nase 1–20	25	—	—	300
Nase 11–30	150	—	—	450
Nase 21–40	—	—	—	—
Nase 31–50	—	—	—	500
Nase 41–60	—	—	—	—
Nase 51–70	—	—	—	—
Nase 61–80	225	—	—	500
Nase 71–90	—	—	—	—
Nase 81–100	—	—	—	25
Nase 91–110	125	—	65	—
Nase 101–120	20	500	400	35
Nase 112–130	—	150	550	350
Nase 121–140	—	—	—	125
Nase 131–149	—	—	—	—

Ia-binding site present on both peptides. If this were the case in all instances of adjacent peptide binding to the same MHC, then there would be three peptide binding regions for IA^d, one for IE^d, two for IA^k and six for IE^k.

We next studied the capacity of each of the 14 Nase peptides to immunize BALB/c(IA^d, IE^d) and CBA/J (IA^k,IE^k) mice (table 3). For those peptides that were immunogenic, the MHC restriction element used in the immune response was determined by anti-Ia inhibition or by immunization of congenic strains of mice. In BALB/c mice, three peptides (1–20, 61–80 and 101–120) were found to be immunogenic. All three peptides were restricted to IA^d, and for one

Table 3. Immunogenicity of the Nase peptides

(Mice were immunized with 25 μg of the different Nase peptides in complete Freund's adjuvant. One week later lymph node cells were obtained and T cells nylon-wool purified. In triplicate microtitre plates, 4×10^5 T cells per well were incubated with 2×10^5 spleen cells, irradiated at 4000 R,[a] per well and 1 μg of peptide. [^{3}H]TdR incorporation was measured five days later by scintillation spectrometry. The mean responses of two or more experiments are reported with the background (T cells plus spleen cells without antigen) subtracted. NT, not tested.)

Nase peptide	mean 3H-TdR incorporation × 10^{-3} by T cells		
	BALB/c	CBA/J	BIO.A (4R)
Nase 1–20	57	25	1
Nase 11–30	−1	15	NT
Nase 21–40	3	4	NT
Nase 31–50	−1	9	NT
Nase 41–60	0	0	NT
Nase 51–70	0	1	NT
Nase 61–80	90	−1	NT
Nase 71–90	0	−2	NT
Nase 81–100	6	107	0
Nase 91–110	2	70	33
Nase 101–120	61	71	27
Nase 112–130	5	110	32
Nase 121–140	1	50	1
Nase 131–149	1	−3	NT

[a] 1 R = 2.58×10^{-4} C kg^{-1}.

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peptide, Nase 101–120, IE^d was also used as a restriction element. For CBA/J mice, seven peptides, 1–20, 11–30, 81–100, 91–110, 101–120, 112–130 and 121–140, were immunogenic. By their failure to be immunogenic in BIO.A(4R) mice, it could be concluded that three of these peptides were restricted solely by IE^k (Nase 1–20, 81–100 and 121–140). Both IE^k and IA^k were used as restriction elements, roughly equally, for Nase 91–110 and 112–130; and for 101–120, IE^k was used as the major restriction element, together with a minor IA^k component. Owing to the relatively low response, the restriction element used by Nase 11–30 could not be determined.

Finally, the data on the capacity of the four Ia molecules to bind the 14 Nase peptides was combined with the data on the ability of the different Ia specificities to serve as restriction elements for Nase-specific T-cell responses. The level at which binding of a peptide to Ia was significant with respect to immunogenicity was not known, but empirically it was observed that all combinations of Nase peptides and Ia that resulted in very weak or undetectable binding (50% inhibitory concentration over 600 μM), with one exception did not stimulate a T-cell response. Using this as a lower limit of 'significant binding,' we assessed the relation between binding and immunogenicity. Seventeen of the 54 combinations tested (31%) showed significant binding to Ia; of those, a T-cell response was elicited in twelve (70%) instances. In only one instance did a peptide that failed to bind to Ia at this level induce a T-cell response (Nase 91–110/IE^k).

The finding of a significant correlation between peptide binding to Ia and immunogenicity strongly supports the determinant selection hypothesis, i.e. that Ia serves as a receptor that selects antigenic determinants and that binding of antigen to Ia is a prerequisite for T-cell recognition. Assuming that the staphylococcal nuclease peptides are representative of other protein antigens, the data would suggest that only about 30% of peptide/Ia interactions are of an affinity compatible with immunogenicity. That is, at the level of any single MHC specificity, less than one third of the potential antigenic determinant are selected for presentation. Furthermore, of those peptides that have been selected on the basis of their capacity to interact with MHC, only about 70% induce a T-cell response, leaving the other 30% as presumed 'holes in the T-cell repertoire.' Thus we conclude that both determinant selection and holes in the T-cell repertoire act in concert to define the immune responsiveness of an individual.

The significance of peptide/Ia interaction in terms of what strength of binding is needed to allow presentation to T cells had been hitherto unknown. In a previous study of a panel of peptides that had been selected for their immunogenicity, we found that most of these peptides bound to their restriction element with a 50% inhibitory concentration of 5–100 μM (Buus *et al.* 1987*a*). In table 4, the binding of the Nase peptides to Ia has been divided into three groups: 50% inhibition at 5–100 μM, at 101–600 μM, and over 600 μM, and the T-cell stimulatory capacity of these groups determined. Five of five peptides that bound to Ia at a 50% inhibitory concentration of 5–100 μM were capable of eliciting T-cell responses, whereas only seven of the 12 peptides (58%) that bound to Ia at 50% inhibitory concentrations between 101 and 600 μM were capable of doing so; none of the peptides with a 50% inhibitory concentration over 600 μM elicited an immune response. Thus the affinity of a peptide for Ia had a profound influence on its T-cell stimulatory capacity. The great majority of the best interactions are productive, dropping off to less than half of the interactions with intermediate affinity and disappearing completely with the lowest affinity. These data suggest that defects in the T-cell

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repertoire are most pronounced for peptides with intermediate affinity for Ia and are rarely seen for the best Ia-binding peptides (we have previously observed one instance of a strong binding peptide to a given Ia that failed to be immunogenic in the context of that restriction element) (Guillet *et al.* 1987).

Although the mechanism behind this association of intermediate affinity of MHC binding and the high incidence of defects in the T-cell response is unknown, it would be anticipated that a high-affinity interaction would result in larger numbers of MHC-antigen complexes being present on the surface of an antigen-presenting cell, thus inducing more T-cell clonotypes than lower-affinity interactions. Consequently, defects in the T-cell repertoire would be less likely to be observed for high-affinity antigen/Ia interactions, as a greater number of 'holes in the repertoire' would have to be present for a non-responder status to be observed.

Table 4. Correlation between binding to Ia and immunogenicity

binding to Ia (50% inhibition dose)	immunogenicity yes	no	total
5–100 μM	5	0	5
101–600 μM	7	5	12
> 600 μM	1	36	37
total	13	41	54

On a population basis, Ia molecules are among the most polymorphic proteins known; however, each individual possesses only a few Ia alleles. Studies of binding of peptides to Ia have suggested that each Ia molecule possesses a single binding site. How does the immune system achieve a sufficient T-cell repertoire despite the requirements for specific binding of antigen to Ia? We have previously found each Ia can bind many seemingly different peptides and that Ia is very permissive in its capacity to bind antigen, probably because it recognizes broadly defined 'motifs' within antigens (Sette *et al.* 1988). Indeed, the data presented herein demonstrate that of the 14 peptides representing the Nase protein, five bound to IA^d with a 50% inhibition dose of 600 μM or less, two to IE^d, three to IA^k and eight to IE^k. Because one binding site might appear in two adjacent peptides, a minimum of three sites were detected for IA^d, one for IE^d, one for IA^k and six for IE^k. Thus, on average, an Ia specificity bound three peptide regions within the Nase protein. By extrapolation it can be estimated that each Ia specificity will bind approximately 18 sites on a protein antigen of 100 kDa. Thus by using an unbiased panel of peptides it does not appear likely that even the smallest micro-organism with only a few proteins could escape the immune system owing to the absence of Ia binding sites.

References

Allen, P. M., Babbitt, B. & Unanue, E. 1987 T cell recognition of lysozyme – the biochemical basis of presentation. *Immunol. Rev.* **98**, 172.

Babbitt, B., Allen, P., Matsueda, G., Haber, E. & Unanue, E. 1985 The binding of immunogenic peptides to Ia histocompatibility molecules. *Nature, Lond.* **317**, 359–361.

Buus, S., Sette, A., Colon, S. M., Jenis, D. M. & Grey, H. M. 1986 Isolation and characterization of antigen–Ia complexes involved in T cell recognition. *Cell* **47**, 1071–1077.

Buus, S., Sette, A., Colon, S., Miles, C. & Grey, H. M. 1987*a* The relationship between major histocompatibility complex (MHC) restriction and the capacity of Ia to bind immunogenic peptides. *Science, Wash.* **235**, 1353–1358.

Buus, S., Sette, A. & Grey, H. M. 1987*b* The interaction between protein derived immunogenic peptides and Ia. *Immunol. Rev.* **98**, 115–141.

Downloaded from rstb.royalsocietypublishing.org

Finnegan, A., Smith, M. A., Smith, J. A., Berzofsky, J., Sachs, D. H. & Hodes, R. J. 1986 The T cell repertoire for recognition of a phylogenetically distant protein antigen. *J. exp. Med.* **164**, 897.

Grantham, R. 1974 Amino acid difference formula to help explain protein evolution. *Science, Wash.* **185**, 862–864.

Guillet, J., Lai, M., Briner, T. J., Buus, S., Sette, A., Grey, H., Smith, J. & Gefter, M. 1987 Immunological self, nonself discrimination. *Science, Wash.* **235**, 1353.

Klein, J. 1982 The major histocompatibility complex. In *Immunology*, pp. 299–302. New York: John Wiley.

Padlan, E. A. 1977 Structural implications of sequence variability in immunoglobulins. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2551–2555.

Paul, W. 1984 Immune response genes. In *Fundamental immunology* (ed. W. Paul), pp. 439–455. New York: Raven Press.

Rosenthal, A. S. 1978 Determinant selection and macrophage function in genetic control of the immune response. *Immunol. Rev.* **40**, 136–152.

Sette, A. S., Buus, S., Colon, S., Miles, C. & Grey, H. 1988 I-A^d binding peptides derived from unrelated protein antigens share a common structural motif. *J. Immunol.* **141**, 45–48.

Shimonkevitz, R., Kappler, J., Marrack, P. & Grey, H. 1983 Antigen recognition by H-2 restricted T cells. I. Cell free antigen processing. *J. exp. Med.* **158**, 303–316.

Watts, T. H., Brian, A. A., Kappler, J. W., Marrack, P. & McConnell, H. M. 1984 Antigen presentation by supported planar membranes containing affinity purified I-A^d. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7564–7568.

Werdelin, O. J. 1982 Chemically related antigens compete for presentation by accessory cells to T cells. *J. Immunol.* **129**, 1883.

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