Review

Antigen Processing by Autoreactive B Cells Promotes Determinant Spreading

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Acute primary immune responses tend to focus on few immunodominant determinants using a very limited number of T cell clones for expansion, whereas chronic inflammatory responses generally recruit a large number of different T cell clones to attack a broader range of determinants of the invading pathogens or the inflamed tissues. In T cell-mediated organ-specific autoimmune disease, a transition from the acute to the chronic phase contributes to pathogenesis, and the broadening process is called determinant spreading. The cellular components catalyzing the spreading reaction are not identified. It has been suggested that autoreactive B cells may play a central role in diversifying autoreactive T cell responses, possibly through affecting antigen processing and presentation. The clonal identity and diversity of the B cells and antibodies seem critical in regulating T cell activity and subsequent tissue damage or repair. Here, we use two autoimmune animal models, experimental autoimmune thyroiditis (EAT) and type 1 diabetes (T1D), to discuss how autoreactive B cells or antibodies alter the processing and presentation of autoantigens to regulate specific T cell response. *Cellular & Molecular Immunology*. 2005;2(3):169-175.

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Immunodominance and determinant spreading

Antigen processing selects immunodominant targets

The primary immune responses against self antigens (Ag) tend to focus on one or few regions, using a very limited number of the available T cell clones (1, 2). There is competition among the T cells responding to the few peptide-MHC complexes, and only clones with the highest affinity to the complexes predominate. This phenomenon is called immunodominance, a term also used to describe a similar initial focusing activity of the host immune system against specific determinants of foreign Ags (3, 4). To become an immunodominant target of a T cell response, a short peptide has to satisfy at least two basic requirements: 1) it must be readily generated from intact Ag and bind to MHC molecules; and 2) it should form a peptide-MHC complex that is "visible" to the existing T cell repertoire. Structural constraints of the native Ag -- its sites of initial enzymatic cleavage by endoproteases and the relative MHC loading efficiency of the products of the initial cleavage, as dictated by their relative MHC-binding affinity, influence the choice of dominant determinants. Different APC populations vary in endoprotease content, and their proteolytic effectiveness is also affected by various maturation and/or activation stimuli (5, 6). An elegant study by Mellman and his colleagues demonstrated that lysosomal acidification and subsequent increase of proteolytic activity during the maturation of dendritic cells are responsible for an enhanced presentation of peptide-MHC complexes (7). MHC-guided processing. i.e., additional trimming of MHC-bound peptides after initial enzymatic cleavage may also be required for generation of best-fit T cell ligands (4, 8). The open ends of the peptide-binding groove of class II MHC molecules allow peptides with various lengths to be presented. Accordingly, dominantly presented peptides eluted from MHC II are not restricted in their length but rather present as nested sets, and each set shares an identical, MHC-binding, core sequence with various number of flanking residues (9-11). It has been suggested that these nested sets are created possibly by specific protease cleavage at the C-terminus and random trimming at the N-terminus which seems driven by the proximity of the N-terminal flanking residues to the core sequences (12). The immunological significance of these

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nested sets is unknown although crystal structure analysis has suggested that the flanking region of an I-A^u determinant, MBP1-11, could greatly affect the binding affinity of the peptide to the MHC molecule (13).

Effects of TCR-ligand affinity on immunodominance

For many years, a central debate has continued over which factors – MHC – peptide binding or affinity-dependent T cell clonal selection, contributes most to immunodominance. There is no doubt that immunodominant peptides must have a high affinity for MHC molecules. Within a competent T cell repertoire, there are many T cells that can recognize a single dominant target, possibly with a range of various affinities for the dominant target and to some mimicking peptide-MHC complexes. In an immune response against foreign Ags, TCR affinity for the peptide-MHC complexes should be the driving force in selecting which T cell clone(s) will be dominant. However, in autoimmunity, those T cells with the highest affinity for dominant self targets are eliminated during thymic selection. Surprisingly, many pathogenic autoreactive T cell clones have a high affinity for particular self determinants. One plausible explanation is that dominant targets of self Ags may remain cryptic under normal conditions, either through an insufficient processing and presentation (4), or by competition from overlapping higher affinity MHC-binding determinants (14-16), which may cause a shift of anchoring to MHC groove. Competition among T cells could also be affected by an intrinsic structural interference of the flanking residues. This competition within dominant regions of self Ags for MHC binding and/or TCR recognition seems reasonable since current evidence supports that the mature TCR repertoire is heavily biased toward recognizing dominant self molecules owing to the requirement of an interaction with self determinants during positive selection of T cells in the thymus (17, 18).

Determinant spreading

If the immunodominant response fails to clear the targets at first, the immune system will mount a more diversified and possibly long-lasting inflammatory response locally or systemically. This process of broadening the initially restricted immune response is called determinant spreading (19). Spreading can occur within a single molecule (intramolecular) or among different nearby molecules (intermolecular) (20). Unlike the immunodominant response, where regulation of the few dominant "driver" T cell clones would be very efficient (21), a spreaded T cell response would be more difficult to regulate due to the increased TCR diversity among the effector T cells. If coupled with malfunctioning regulatory component(s), which are crucial for the down-regulation of the dominant T cells, a chronic autoimmune response would lead to irreversible pathogenesis. In fact, most organ-specific autoimmune diseases are linked to multiple genetic components controlling the regulatory network besides MHC molecules (22, 23). Encouragingly, the spreading cascade could be interrupted by the intro-duction of tolerance to dominant determinants at an early stage (24, 25), although the mechanism of spreading is unclear. Nevertheless, a Th1-biased inflammatory environment created during the initial immunodominant, high affinity reaction is apparently indispensable, and Th2 cells are insufficient to counter the dominant Th1 influence (26, 27), possibly due to an insufficient differentiation to the Th2 pathway at an early stage. It is believed that the spreading process involves altered Ag processing and presentation as well as increased costimulation (28).

Antigen-specific processing in B cells and T-B reciprocal activation

B cells are well-known for their dramatic increase in Ag uptake through surface immunoglobulin molecules (sIg) and subsequent enhancement of peptide presentation (29, 30). Antibodies (Ab) can increase the efficiency of Ag capture by 10^3-10^4-fold in a piggy-back manner through FcR-mediated internalization (31), leading to increased Ag delivery to the processing compartment and presentation of MHC-peptide complexes on FcR^+ professional APC (32). However, the increased Ag delivery and presentation is not sufficient to activate native T cells. In fact, oral or *i.v.* introduction of self Ags or their peptides without adjuvant frequently induces tolerance (33). Qualitative changes in Ag processing and additional assistance from costimulation are required to break established tolerance to dominant self determinants. Interestingly, binding sIg to its specific ligand will stimulate B cells to increase the expression of costimulation molecules, e.g., CD40 and B7 (34, 35). Thus, Ag-bound to B cells receptors may provide a unique pathway to activate previously tolerized cognate T cells (Figure 1).

Along with increased presentation of previously tolerized dominant self determinants, B cells could display previous non-tolerized, cryptic self determinants following an altered Ag processing activity. Berzofsky's (36) and Celada's (37, 38) group, and then Lanzavecchia, Watts and their colleagues (39, 40) showed, in elegant experiments, that depending upon which portion of the antigenic molecules was bound by the Ig receptor on the B cell, different T cell determinants on the molecule would be preferentially presented in association with MHC molecule. The relative topology of the different T and B cell epitopes might play an important role in T-B reciprocal activation (37, 40). Mamula reinforced the notion of T-B reciprocal activation in systemic auto-immune disease (41). We first reported this T-B reciprocal activation phenomenon in autoimmunity by demonstrating that autoreactive mAbs against thyroglobulin (Tg) could alter the processing and presentation of a subdominant pathogenic T cell determinant within Tg (42). It is unclear why T cells recognizing the cryptic determinants exist in the peripheral repertoire, provided that a TCR-peptide-MHC interaction is necessary to generate and maintain the repertoire.

Experimental autoimmune thyroiditis (EAT)

What do autoreactive T and B cells recognize?

Patients with autoimmune thyroiditis (AT) frequently develop

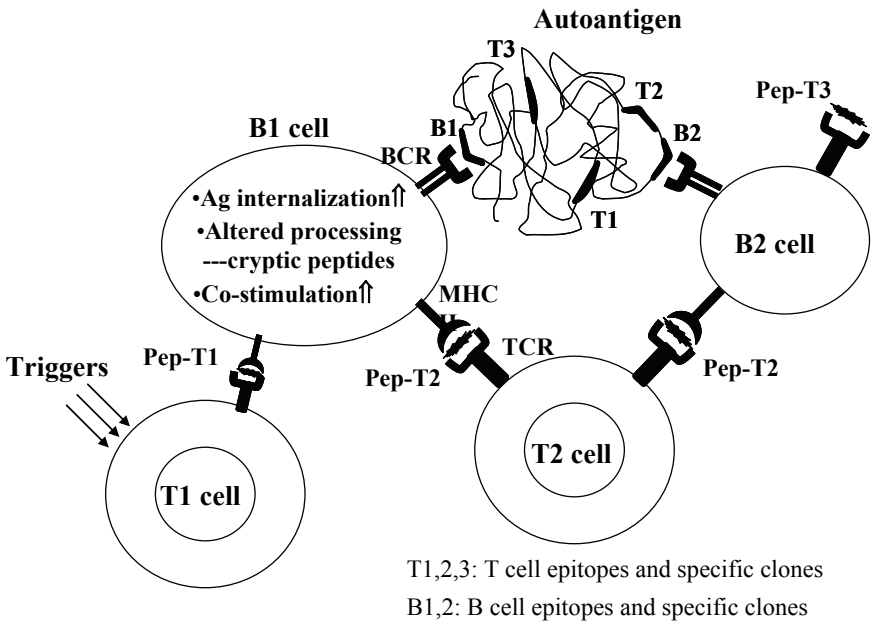

Figure 1. Ag-specific T-B activation cascade during determinant spreading. T-B cognates form after initial triggering of a "driver-like" Th1 T1 cell, whose activity is required to transform antigen-specific B cells as competent effective APC, and thus to initiate the spreading cascade. Three major events may occur to Ag-specific B cells after activation: 1) enhanced Ag internalization by surface Ig; 2) altered delivery of the Ag to special endosomal compartments and differential processing and generation of cryptic determinants such as T2 and T3; and 3) upregulated costimuation such as B7 and CD40.

autoantibodies (auto-Abs) against Tg and thyroid peroxidase (TPO) (43). However, while serum anti-Tg and anti-TPO Abs have been recognized as important indicators for AT diagnosis, their presence does not necessarily indicate occurrence of AT. Depending on the methods used for detection of the serum auto-Abs, the frequency of auto-Ab positive HT patients can vary from 60% to 100% (44). Such a high frequency clearly demonstrates a strong association between the anti-Tg/TPO auto-Abs and the development of AT (45). Currently, it is not clear whether or how these auto-Abs are involved in the pathogenesis of AT. It has been reported that Tg-specific Abs cannot fix complement, but may play a role in antibody-dependent cell-mediated cytotoxicity (ADCC) (46). Successful induction of EAT by transferring sera from Tg-immunized animals to syngeneic recipients has been reported in different animal models (47-49). Also, mice injected with hybridoma cells secreting anti-Tg mAb developed thyroid lesions quickly (50). However, other conflicting studies have shown that Abs or Tg-reactive serum cannot directly induce EAT (51). Healthy people and AT patients carry different types of anti-Tg Abs in their circulation, indicating that some auto-Ab clones may be harmless and others may be pathogenic and related to the development of AT (52). Most disease-associated anti-Tg auto-Abs are oligoclonal and restricted in their epitopic specificity (53, 54), while the natural anti-Tg auto-Abs in healthy people are more likely to be polyclonal (55). In addition, Dong et al. reported that the binding sites of pathogenic anti-Tg Abs are clustered within certain regions of Tg, but the epitopes of the natural anti-Tg Abs are spread randomly within Tg (56). More recently, Prentice et al. have also demonstrated that the Tg epitopes recognized by Tg-specific Abs from AT patients are different from those recognized by Abs from healthy individuals (57).

Various strategies have been used for mapping T cell determinants critical for EAT development. Champion et al. found that poorly iodinated Tg failed to elicit EAT in mice and could not activate Tg-specific T-cell hybridomas (58). This observation raised the possibility that dominant pathogenic Tg epitopes might be iodinated. T-cell epitope mapping efforts led to the identification of a thyroiditogenic, thyroxine (T4)-containing Tg epitope, T4(2553), which contains a thyroxine molecule at position 2553 (59, 60). This study emphasized a role for peptides encompassing hormonogenic sites in EAT pathogenesis. The use of computerized algorithms searching for MHC-binding motifs in Tg allowed the identification of several other pathogenic Tg peptides: 306-320, 1579-1591, 1826-1836, 2102-2116, 2495-2511, 2596-2608 and 2694-2711, as evidenced by their capability of inducing specific T cells that can infiltrate the thyroid glands (61-63). All these peptides encompassed non-dominant T-cell epitopes, since they could not stimulate Tg-primed LNC *in vitro*, and they could not prime T-cells able to recognize intact Tg *in vitro* (64, 65). Immunodominant

determinant(s) in Tg has not yet been identified.

Enhancement or inhibition of Tg peptide-specific T cell responses by anti-Tg Abs

We have generated T-cell hybridoma clones against two pathogenic MHC class II-binding peptides at the C-terminal end of Tg: a subdominant peptide (2549-2560) which is derived following processing of Tg *in vivo* but not *in vitro* (60, 66); and a cryptic peptide (2495-2511), which is not generated following processing of intact Tg either *in vivo* or *in vitro* (62). To examine whether anti-Tg mAbs bound to Tg would interfere with Tg processing by APC, various Tg-mAbs immune complexes (IC) were pre-formed and then used to pulse APC. Presentation of the two peptides on the IC-pulsed APC was evaluated by activation of the appropriate T cell hybridomas. We found that generation of the T4(2553) peptide is augmented by two Tg-specific IgG mAbs which facilitated FcR-mediated internalization of Tg. However, other mAbs, of the same (IgG1) subclass, similarly enhanced Tg uptake by APC, but had no effect on the generation of this peptide (42). The boosting effect was selective since the enhancing mAbs did not facilitate generation of the neighboring cryptic (2495-2511) peptide. When Tg was simultaneously complexed to a mAb reactive with T4(2553) and to a mixture of boosting mAbs, the presentation of this epitope was completely suppressed. These results suggested that Tg-specific antibodies alter Tg processing and may boost or suppress the presentation of subdominant pathogenic determinants during the course of disease.

Type 1 diabetes (T1D)

Clinical relevance of anti-GAD, insulin and IA-2 antibodies

Glutamic acid decarboxylase (GAD)-65 (67), insulinoma-associated protein tyrosine phosphatase-2 (IA-2) (68) and insulin (69) are the three major auto-Ags related to T1D. Auto-Abs against these Ags can be detected long before the appearance of clinical symptoms, suggesting that the immune pathogenic response starts early and spontaneously. Therefore, it is possible to use auto-Abs to predict the occurrence of T1D. Efforts to study these antibodies and their correlation to T1D have been taken, trying to standardize a protocol for the purpose of prediction or diagnosis (70). Over 90% of newly diagnosed subjects have auto-Abs against one or more of the three Ags. In general, anti-GAD antibodies are stable through the course of disease, whereas anti-IA-2 antibodies tend to decrease with the duration of disease, and few subjects display anti-insulin antibodies although age and insulin treatment may have effects on the titer of anti-insulin antibodies. Interestingly, it seems that the diversity or the number of different autoantibodies is more important than the titer of antibodies specific for an individual Ag with respect to the correlation to the development of diabetes (71), indicating a critical pathogenic activity of determinant spreading.

B cells function as APC in T1D and T-B reciprocal interaction

B cells are necessary for development of diabetes in the non-obese diabetic (NOD) mice (72). Capture of autoantigens such as GAD65 by surface immunoglobulin (Ig) is followed by processing and presentation of T cell determinants by B cells, a step that is crucial for activation of autoreactive T cells and induction of diabetes (73-75). B cell-mediated processing of self Ags may contribute to generation of an inflammatory microenvironment in the pancreas, which is critical for overcoming the regulatory barrier(s) of initiation of diabetes in NOD mice (76). Nepom first reported that GAD65-specific mAbs act in a piggy-back manner, through surface FcR-mediated Ag internalization pathway, to boost presentation of dominant T cell determinants on APC after formation of IC with GAD65 (77). Baekkeskov and her colleagues proposed a topological relationship between T cell and B cell determinants in that T cell determinants within the Ig-footprint would be suppressed in the processing machinery (78). Therefore, dominant T cell expansions could be chosen based on where dominant Ab response is initiated within the same auto-Ag. It would be interesting to see whether a B cell epitope hierarchy exists in correlation with the spreading hierarchy in the T cell response.

Activation of diabetic T cells by GAD65-primed APC

Many T cell clones have been isolated from diabetic NOD mice. GAD65 is obviously one of the major targets recognized by the diabetic T cells in the cascade that leads to T1D. NOD mice exhibit a "spontaneous" proliferative response to GAD65 determinants that arises concomitantly with the onset of insulitis (between 4-8 weeks) (2, 79). The autoaggressive response to β cell Ags initially directed toward a few determinants within GAD65 and later spread both intramolecularly and intermolecularly to other candidate diabetogenic autoantigens. The greatest proliferative response is initially directed against determinants contained within p509-528 and p524-543, which later spreads to other regions of the GAD65 molecule (including determinants within p78-97, p246-266, p340-356, p479-493, p540-556 and p570-585), the so-called "spontaneous determinants" of GAD65 (80). A second set of GAD65 CD4 T cell-inducing determinants in NOD mice includes p206-220, p221-235, p286-300 and p400-415-the so-called "immunizable" determinants (81). It is unknown what mechanism drives the selection of different targets within GAD65 during the spontaneous versus the immunized responses. Several lines of evidence implicate the GAD65 peptide, p524-543, as a specific, possibly low affinity stimulus for the spontaneously arising, diabetogenic T cell clone, BDC2.5 (82) (Dai et al., in press). Interestingly, BDC2.5 T cells, which normally are unresponsive to p524-543 stimulation, react to the peptide when provided with splenic APC obtained from mice immunized with the same peptide, p524-543, but not, for example, with hen's egg lysozyme (HEL) (Dai et al., in press). Immunization with p524-543 increases the susceptibility of NOD mice to diabetes induced by the adoptive transfer of BDC2.5 T cells. In addition, very few CFSE-dye-labeled BDC2.5 T cells divide in the recipient's

pancreas following transfer into a transgenic mouse which overexpresses GAD-65 in B cells, while they divide vigorously in the pancreas of normal NOD recipients (Dai et al., unpublished). These data suggest that specific and altered processing of self Ags may play an essential role in the development and expansion of autoreactive T cells.

Conclusion and future prospects

The immune system appears to have two opposite tendencies, to focus its energy on a few antigenic targets in the early stages of an immune response, and to diversify its influence to many enemy targets, late in the response. On one hand, a few highest affinity T cells are selected to attack the strongest antigenic part(s) of pathogens trying to halt the invasion at the first encounter. On the other hand, due to determinant spreading, many different T cells are recruited to fight persistent Ags in chronic inflammation. Alternatively, these two types of responses could occur in a *Yin and Yang* manner, in which focusing and diversification mutually compensate for, or maybe counteract each other in order to sustain the immune response. Ag-specific B cells and their antibodies are essential in catalyzing determinant spreading reaction *via*: a) generation of novel – previously cryptic – epitopes through altered antigen processing or b) facilitation of T cell activation through generation of ligands with higher affinity for TCR and delivery of costimulatory signals. Ag processing through surface receptor-mediated internalization, e.g., FcR and sIg, is different from that occurring through pinocytosis and phagocytosis, but the molecular mechanisms remain unsolved. Signals controlling receptor internalization, recycling and degradation are a major focus of current studies.

References

1. Urban JL, Kumar V, Kono DH, et al. Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy. Cell. 1988;54:577-592.
2. Kaufman DL, Clare-Salzler M, Tian J, et al. Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. Nature. 1993;366:69-72.
3. Katz ME, Maizels RM, Wicker L, Miller A, Sercarz EE. Immunological focusing by th mouse major histocompatibility complex: mouse strains confronted with distantly related lysozymes confine their attention to very few epitopes. Eur J Immunol. 1982;12:535-540.
4. Sercarz EE, Lehmann PV, Ametani A, Benichou G, Miller A, Moudgil K. Dominance and crypticity of T cell antigenic determinants. Annu Rev Immunol. 1993;11:729-766.
5. Schneider SC, Sercarz EE. Antigen processing differences among APC. Hum Immunol. 1997;54:148-158.
6. Delamarre L, Holcombe H, Mellman I. Presentation of exogenous antigens on major histocompatibility complex (MHC) class I and MHC class II molecules is differentially regulated during dendritic cell maturation. J Exp Med. 2003;198:111-122.
7. Trombetta ES, Ebersold M, Garrett W, Pypaert M, Mellman I. Activation of lysosomal function during dendritic cell maturation. Science. 2003;299:1400-1403.
8. Sercarz EE, Maverakis E. MHC-guided processing: binding of large antigen fragments. Nat Rev Immunol. 2003;3:621-629.
9. Rudensky A, Preston-Hurlburt P, Hong SC, Barlow A, Janeway CA, Jr. Sequence analysis of peptides bound to MHC class II molecules. Nature. 1991;353:622-627.
10. Hunt DF, Michel H, Dickinson TA, et al. Peptides presented to the immune system by the murine class II major histocompatibility complex molecule I-Ad. Science. 1992;256:1817-1820.
11. Chicz RM, Urban RG, Lane WS, et al. Predominant naturally processed peptides bound to HLA-DR1 are derived from MHC-related molecules and are heterogeneous in size. Nature. 1992;358:764-768.
12. Lippolis JD, White FM, Marto JA, et al. Analysis of MHC class II antigen processing by quantitation of peptides that constitute nested sets. J Immunol. 2002;169:5089-5097.
13. He XL, Radu C, Sidney J, Sette A, Ward ES, Garcia KC. Structural snapshot of aberrant antigen presentation linked to autoimmunity: the immunodominant epitope of MBP complexed with I-Au. Immunity. 2002;17:83-94.
14. Maverakis E, Beech J, Stevens DB, et al. Autoreactive T cells can be protected from tolerance induction through competition by flanking determinants for access to class II MHC. Proc Natl Acad Sci U S A. 2003;100:5342-5347.
15. Quinn A, McInerney B, Reich EP, Kim O, Jensen KP, Sercarz EE. Regulatory and effector CD4 T cells in nonobese diabetic mice recognize overlapping determinants on glutamic acid decarboxylase and use distinct Vβ genes. J Immunol. 2001; 166:2982-2991.
16. Seamons A, Sutton J, Bai D, et al. Competition between two MHC binding registers in a single peptide processed from myelin basic protein influences tolerance and susceptibility to autoimmunity. J Exp Med. 2003;197:1391-1397.
17. Anderson MS, Venanzi ES, Klein L, et al. Projection of an immunological self shadow within the thymus by the aire protein. Science. 2002;298:1395-1401.
18. Janeway CA, Jr. The role of self-recognition in receptor repertoire development. Members of the Janeway Laboratory. Immunol Res. 1999;19:107-118.
19. Lehmann PV, Forsthuber T, Miller A, Sercarz EE. Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. Nature. 1992;358:155-157.
20. Lehmann PV, Sercarz EE, Forsthuber T, Dayan CM, Gammon G. Determinant spreading and the dynamics of the autoimmune T-cell repertoire. Immunol Today. 1993;14:203-208.
21. Sercarz E, Maverakis E, van den Elzen P, Madakamutil L, Kumar V. Seven surprises in the TCR-centred regulation of immune responsiveness in an autoimmune system. Novartis Found Symp. 2003;252:165-171; discussion 171-166, 203-110.
22. Ueda H, Howson JM, Esposito L, et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature. 2003;423:506-511.
23. Wicker LS, Chamberlain G, Hunter K, et al. Fine mapping, gene content, comparative sequencing, and expression analyses support Ctla4 and Nramp1 as candidates for Idd5.1 and Idd5.2 in the nonobese diabetic mouse. J Immunol. 2004;173:164-173.
24. McRae BL, Vanderlugt CL, Dal Canto MC, Miller SD. Functional evidence for epitope spreading in the relapsing pathology of experimental autoimmune encephalomyelitis. J Exp Med. 1995;182:75-85.
25. Yu M, Johnson JM, Tuohy VK. A predictable sequential determinant spreading cascade invariably accompanies progression of experimental autoimmune encephalomyelitis: a basis for peptide-specific therapy after onset of clinical disease.

J Exp Med. 1996;183:1777-1788.
26. Tian J, Lehmann PV, Kaufman DL. Determinant spreading of T helper cell 2 (Th2) responses to pancreatic islet autoantigens. J Exp Med. 1997;186:2039-2043.
27. Tisch R, Wang B, Atkinson MA, Serreze DV, Friedline R. A glutamic acid decarboxylase 65-specific Th2 cell clone immunoregulates autoimmune diabetes in nonobese diabetic mice. J Immunol. 2001;166:6925-6936.
28. Nikcevich KM, Gordon KB, Tan L, et al. IFN-γ-activated primary murine astrocytes express B7 costimulatory molecules and prime naive antigen-specific T cells. J Immunol. 1997;158: 614-621.
29. Snider DP, Segal DM. Efficiency of antigen presentation after antigen targeting to surface IgD, IgM, MHC, FcγRII, and B220 molecules on murine splenic B cells. J Immunol. 1989;143:59-65.
30. Franco A, Appella E, Kagnoff MF, et al. Peripheral T cell response to A-gliadin in celiac disease: differential processing and presentation capacities of Epstein-Barr-transformed B cells and fibroblasts. Clin Immunol Immunopathol. 1994;71:75-81.
31. Amigorena S, Bonnerot C. Role of B-cell and Fc receptors in the selection of T-cell epitopes. Curr Opin Immunol. 1998;10: 88-92.
32. Watts C. Capture and processing of exogenous antigens for presentation on MHC molecules. Annu Rev Immunol. 1997;15: 821-850.
33. Tian J, Olcott A, Hanssen L, Zekzer D, Kaufman DL. Antigen-based immunotherapy for autoimmune disease: from animal models to humans? Immunol Today. 1999;20:190-195.
34. Noelle RJ, Roy M, Shepherd DM, Stamenkovic I, Ledbetter JA, Aruffo A. A 39-kDa protein on activated helper T cells binds CD40 and transduces the signal for cognate activation of B cells. Proc Natl Acad Sci U S A. 1992;89:6550-6554.
35. Roth R, Nakamura T, Mamula MJ. B7 costimulation and autoantigen specificity enable B cells to activate autoreactive T cells. J Immunol. 1996;157:2924-2931.
36. Berzofsky JA. T-B reciprocity. An Ia-restricted epitope-specific circuit regulating T cell-B cell interaction and antibody specificity. Surv Immunol Res. 1983;2:223-229.
37. Celada F, Sercarz EE. Preferential pairing of T-B specificities in the same antigen: the concept of directional help. Vaccine. 1988;6:94-98.
38. Manca F, Kunkl A, Fenoglio D, Fowler A, Sercarz E, Celada F. Constraints in T-B cooperation related to epitope topology on E. coli beta-galactosidase. I. The fine specificity of T cells dictates the fine specificity of antibodies directed to conformation-dependent determinants. Eur J Immunol. 1985;15:345-350.
39. Simitsek PD, Campbell DG, Lanzavecchia A, Fairweather N, Watts C. Modulation of antigen processing by bound antibodies can boost or suppress class II major histocompatibility complex presentation of different T cell determinants. J Exp Med. 1995; 181:1957-1963.
40. Watts C, Lanzavecchia A. Suppressive effect of antibody on processing of T cell epitopes. J Exp Med. 1993;178:1459-1463.
41. Shlomchik MJ, Craft JE, Mamula MJ. From T to B and back again: positive feedback in systemic autoimmune disease. Nat Rev Immunol. 2001;1:147-153.
42. Dai Y, Carayanniotis KA, Eliades P, et al. Enhancing or suppressive effects of antibodies on processing of a pathogenic T cell epitope in thyroglobulin. J Immunol. 1999;162:6987-6992.
43. Weetman AP, McGregor AM. Autoimmune thyroid disease: developments in our understanding. Endocr Rev. 1984;5:309-355.
44. Bigazzi PE. Autoimmunity in Hashimoto's disease. In: C.A. Bona KAS, M. Zanetti, and A.N. Theofilopoulos, ed. Molecular pathology of autoimmune disease. Langhorne: Harwood Academic; 1993:493-510.
45. Burek CL, Bresler HS. Human autoimmune thyroid disease: risk factors. In: Bigazzi PE, Wick G, Wicher K., ed. Organ-specific autoimmunity. New York and Basel: Marcel Dekker, Inc.; 1990:169-190.
46. Weetman AP, Black CM, Cohen SB, Tomlinson R, Banga JP, Reimer CB. Affinity purification of IgG subclasses and the distribution of thyroid auto-antibody reactivity in Hashimoto's thyroiditis. Scand J Immunol. 1989;30:73-82.
47. Polley CR, Bacon LD, Rose NR. Spontaneous autoimmune thyroiditis in chickens. I. Effects of bursal reconstitution. J Immunol. 1981;127:1465-1468.
48. Tomazic V, Rose NR. Autoimmune murine thyroiditis VII: induction of the thyroid lesions by passive transfer of immune serum. Clin Immunol Immunopathol. 1975;4:511-518.
49. Nakamura RM, Weigle WO. Transfer of experimental autoimmune thyroiditis by serum from thyroidectomized donors. J Exp Med. 1969;130:263-285.
50. Yokochi T, Inoue Y, Fukada M, et al. Histological and functional changes in the thyroid glands of mice implanted with hybridomas secreting monoclonal autoantibody against mouse thyroglobulin. Autoimmunity. 1991;10:125-131.
51. Inoue K, Niesen N, Milgrom F, Albini B. Transfer of experimental autoimmune thyroiditis by *in situ* perfusion of thyroids with immune sera. Clin Immunol Immunopathol. 1993; 66:11-17.
52. Tomer Y. Anti-thyroglobulin autoantibodies in autoimmune thyroid diseases: cross-reactive or pathogenic? Clin Immunol Immunopathol. 1997;82:3-11.
53. Piechaczyk M, Bouanani M, Salhi SL, et al. Antigenic domains on the human thyroglobulin molecule recognized by auto-antibodies in patients' sera and by natural autoantibodies isolated from the sera of healthy subjects. Clin Immunol Immunopathol. 1987;45:114-121.
54. Ruf J, Carayon P, Sarles-Philip N, Kourilsky F, Lissitzky S. Specificity of monoclonal antibodies against human thyroglobulin; comparison with autoimmune antibodies. EMBO J. 1983;2:1821-1826.
55. Dietrich G, Piechaczyk M, Pau B, Kazatchkine MD. Evidence for a restricted idiotypic and epitopic specificity of anti-thyroglobulin autoantibodies in patients with autoimmune thyroiditis. Eur J Immunol. 1991;21:811-814.
56. Dong Q, Ludgate M, Vassart G. Towards an antigenic map of human thyroglobulin: identification of ten epitope-bearing sequences within the primary structure of thyroglobulin. J Endocrinol. 1989;122:169-176.
57. Prentice L, Kiso Y, Fukuma N, et al. Monoclonal thyroglobulin autoantibodies: variable region analysis and epitope recognition. J Clin Endocrinol Metab. 1995;80:977-986.
58. Champion BR, Rayner DC, Byfield PG, Page KR, Chan CT, Roitt IM. Critical role of iodination for T cell recognition of thyroglobulin in experimental murine thyroid autoimmunity. J Immunol. 1987;139:3665-3670.
59. Champion BR, Page KR, Parish N, et al. Identification of a thyroxine-containing self-epitope of thyroglobulin which triggers thyroid autoreactive T cells. J Exp Med. 1991;174: 363-370.
60. Hutchings PR, Cooke A, Dawe K, et al. A thyroxine-containing peptide can induce murine experimental autoimmune thyroiditis. J Exp Med. 1992;175:869-872.
61. Carayanniotis G, Chronopoulou E, Rao VP. Distinct genetic

pattern of mouse susceptibility to thyroiditis induced by a novel thyroglobulin peptide. Immunogenetics. 1994;39:21-28.
62. Chronopoulou E, Carayanniotis G. Identification of a thyroiditogenic sequence within the thyroglobulin molecule. J Immunol. 1992;149:1039-1044.
63. Verginis P, Stanford MM, Carayanniotis G. Delineation of five thyroglobulin T cell epitopes with pathogenic potential in experimental autoimmune thyroiditis. J Immunol. 2002;169: 5332-5337.
64. Carayanniotis G, Rao VP. Searching for pathogenic epitopes in thyroglobulin: parameters and caveats. Immunol Today. 1997; 18:83-88.
65. Carayanniotis G. The cryptic self in thyroid autoimmunity: the paradigm of thyroglobulin. Autoimmunity. 2003;36:423-428.
66. Wan Q, Motte RW, McCormick DJ, et al. Primary hormonogenic sites as conserved autoepitopes on thyroglobulin in murine autoimmune thyroiditis: role of MHC class II. Clin Immunol Immunopathol. 1997;85:187-194.
67. Baekkeskov S, Aanstoot HJ, Christgau S, et al. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature. 1990;347:151-156.
68. Lan MS, Wasserfall C, Maclaren NK, Notkins AL. IA-2, a transmembrane protein of the protein tyrosine phosphatase family, is a major autoantigen in insulin-dependent diabetes mellitus. Proc Natl Acad Sci U S A. 1996;93:6367-6370.
69. Eisenbarth GS, Moriyama H, Robles DT, et al. Insulin autoimmunity: prediction/precipitation/prevention type 1A diabetes. Autoimmun Rev. 2002;1:139-145.
70. Leslie D, Lipsky P, Notkins AL. Autoantibodies as predictors of disease. J Clin Invest. 2001;108:1417-1422.
71. Notkins AL. Type 1 diabetes as a model for autoantibodies as predictors of autoimmune diseases. Autoimmun Rev. 2004;3 Suppl 1:S7-9.
72. Serreze DV, Chapman HD, Varnum DS, et al. B lymphocytes are essential for the initiation of T cell-mediated autoimmune diabetes: analysis of a new "speed congenic" stock of NOD.Ig mu null mice. J Exp Med. 1996;184:2049-2053.
73. Serreze DV, Fleming SA, Chapman HD, Richard SD, Leiter EH, Tisch RM. B lymphocytes are critical antigen-presenting cells for the initiation of T cell-mediated autoimmune diabetes in nonobese diabetic mice. J Immunol. 1998;161:3912-3918.
74. Falcone M, Lee J, Patstone G, Yeung B, Sarvetnick N. B lymphocytes are crucial antigen-presenting cells in the pathogenic autoimmune response to GAD65 antigen in nonobese diabetic mice. J Immunol. 1998;161:1163-1168.
75. Silveira PA, Johnson E, Chapman HD, Bui T, Tisch RM, Serreze DV. The preferential ability of B lymphocytes to act as diabetogenic APC in NOD mice depends on expression of self-antigen-specific immunoglobulin receptors. Eur J Immunol. 2002;32:3657-3666.
76. Greeley SA, Moore DJ, Noorchashm H, et al. Impaired activation of islet-reactive CD4 T cells in pancreatic lymph nodes of B cell-deficient nonobese diabetic mice. J Immunol. 2001;167:4351-4357.
77. Reijonen H, Daniels TL, Lernmark A, Nepom GT. GAD65-specific autoantibodies enhance the presentation of an immunodominant T-cell epitope from GAD65. Diabetes. 2000; 49:1621-1626.
78. Jaume JC, Parry SL, Madec AM, Sonderstrup G, Baekkeskov S. Suppressive effect of glutamic acid decarboxylase 65-specific autoimmune B lymphocytes on processing of T cell determinants located within the antibody epitope. J Immunol. 2002;169:665-672.
79. Tisch R, Yang XD, Singer SM, Liblau RS, Fugger L, McDevitt HO. Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. Nature. 1993;366:72-75.
80. Quinn A, Sercarz EE. T cells with multiple fine specificities are used by non-obese diabetic (NOD) mice in the response to GAD(524-543). J Autoimmun. 1996;9:365-370.
81. Chao CC, McDevitt HO. Identification of immunogenic epitopes of GAD 65 presented by Ag7 in non-obese diabetic mice. Immunogenetics. 1997;46:29-34.
82. Judkowski V, Pinilla C, Schroder K, Tucker L, Sarvetnick N, Wilson DB. Identification of MHC class II-restricted peptide ligands, including a glutamic acid decarboxylase 65 sequence, that stimulate diabetogenic T cells from transgenic BDC2.5 nonobese diabetic mice. J Immunol. 2001;166:908-917.