

Autoimmunity and asthma

The dirt on the hygiene hypothesis

Mark D. Mannie

The Department of Microbiology and Immunology; Brody School of Medicine; East Carolina University; Greenville, NC USA

Self peptides shape T-cell development through selectional processes in the thymus and secondary lymphoid organs to promote a diverse and balanced repertoire of conventional and regulatory T cells. Foreign proteins and their derivative peptides permeate our mucosal tissues to constitute another diverse array of peptides that may specify and diversify the mucosal T-cell repertoire. Indeed, the distinction between self peptides and environmental foreign peptides may be academic if both are present constantly within the body. The premise here is that the plethora of foreign peptides, present ubiquitously in our environment and body, form homeostatic niches to foster highly diversified repertoires of conventional and regulatory T cells that recognize persistent environmental peptides as self. Highly diversified repertoires that recognize myriads of self and environmental foreign peptides as homeostatic ligands may be critical for adaptive distinctions of friend or foe in mucosal tissues. The change from our agrarian past to the highly sterile environments of today may adversely impact the diversity and concentrations of foreign peptides that shape the mucosal T-cell repertoire. Various hygiene hypotheses postulate that the lack of factors such as infectious pathogens, innate receptor engagement or Th1 bias is key to the marked increase in immunological disease in modern society. In this version of the hygiene hypothesis, highly diverse constellations of innocuous environmental peptides are postulated to be the critical factor for immune balance and homeostasis.

Self Peptides and the Generation of a Highly Diverse T-Cell Repertoire

Thymic differentiation and selection represent an amazing developmental feat. From a genetic program with the input of resources and energy, the thymus generates a clonotypically arrayed, vast assemblage of T-cell antigen receptor (TCR) structures able to recognize a plethora of self peptides on a restricted set of polymorphic MHC class I and II gene products.¹⁻⁶ T-cell recognition of this vast repertoire of self peptide/MHC complexes has been selected and tuned so that each recognition event is a low efficiency interaction that imparts a positive homeostatic signal. These 'weak' signals enable survival and persistence of a given clone. By this process, the T-cell repertoire 'learns' to use the set of inherited MHC molecules to reject dangerous pathogens.

Thymic selection is guided by a set-point theory. Selectional and tuning programs purge the T-cell repertoire of highly reactive T cells having potential for pathogenic autoreactivity. The repertoire is also purged of T cells that have 'useless' TCR which are unlikely to have meaningful cross-reactivity with foreign agonist peptides on host MHC molecules. Positive selection of the repertoire favors a subset of clones that impart a low efficiency signal with each clone having specificity for a limited set of self-MHC ligands. The nature of this low-efficiency, positively-selecting interaction is incompletely understood. The signal qualitatively lacks efficacy—even high concentrations of this ligand will not drive overt blastogenesis and differentiation into

Key words: hygiene hypothesis, autoimmune disease, allergic disease, asthma, regulatory T cells, T-cell repertoire, antigenic peptides

Submitted: 02/12/10

Accepted: 02/15/10

Previously published online:
www.landesbioscience.com/journals/selfnonself/article/11550

Correspondence to:
Mark D. Mannie; Email: manniem@ecu.edu

an effector or memory status. An appropriate set-point is facilitated by mechanisms of selection and receptor tuning. Thymocytes that recognize their ligands as negatively-selecting, strong ligands may avoid apoptosis by developmental programs that damp the strength of TCR-mediated signal transduction pathways. These clones may permanently downregulate co-receptor levels and the strength of TCR signal transduction pathways. These reformed clones may no longer recognize a given ligand as a negative-selecting agonist ligand but rather as a low efficacy, inefficient ligand within an efficacy range that is beneficial and drives positive selection and maintenance of the clone.

Through this process, thymic output is characterized by a self-reinforcing system of homeostatic weak interactions. As the repertoire seeds peripheral tissues and secondary lymphoid organs, the same self-selecting process is evident in the selection and maintenance of the mature T-cell repertoire.^{7,8} Mature naïve T cells may be long-lived with life-spans contingent upon continued clonotypic interactions with the set of positively-selecting ligands first encountered in the thymus. Given the vastness of the T-cell repertoire, some clones will exhibit cross-reactivity between the low-efficacy, positively-selecting, peptide-MHC ligands and high-efficacy foreign peptide-MHC ligands. This cross-reactivity is the basis of host defense. The probability of cross-reactivity is greatly fostered because the same polymorphic MHC structure is the basis for presentation of a homeostatic peptide and the cross-reacting foreign agonist peptide.

The main point is that a vast diversity of self peptides is fundamentally important for shaping the T-cell repertoire. Self peptides thereby represent a critical formative stimulus of a mature T cell repertoire.

Partial Immunodeficiency and Immune Dysregulation

An important clue regarding mechanisms of self tolerance and immunopathogenesis is the unexpected correlation between partial T-cell immunodeficiency and immune dysregulation.⁹⁻¹² Complete T-cell immunodeficiency is clinically rare, represents a qualitative knock-out of a gene function,

and fully disables adaptive immunity. In contrast, partial T-cell deficiency represents a more common clinical scenario that reflects a quantitative impairment of a gene product and diminished adaptive immune responses. Patients with a wide variety of different partial immunodeficiency diseases co-present with allergic hypersensitivity and/or autoimmune disease.⁹ The paradox is that a partial T-cell immunodeficiency would be predicted to confer hypo-reactive immunity. However, as many as 50% of patients with partial T-cell deficiencies present with autoimmune disease or hypersensitivity. These include primary immunodeficiency diseases, representing partial defects in genes such as RAG1, RAG2, LIG4, ARTEMIS, ADA, ZAP70 or STAT5B that cause a secondary autoimmunity. This striking predilection excludes defects in known T-cell regulatory pathways such as those underlying IPEX and APECED, representing defects in regulatory genes such as Foxp3 or AIRE, which cause primary autoimmune diseases that in turn result in a secondary immunodeficiency.

A wide variety of gene knock-downs also result in partial T-cell deficiency in mouse models, and many of these gene defects are also associated with autoimmunity and hypersensitivity.⁹ The wide array of gene defects that reveal this association lends credence to a possible causal relation between partial immunodeficiency and immune dysregulation. The Omenn syndrome for example, when present as a partial immunodeficiency of V-D-J recombination, results in the emergence of a limited oligoclonal T-cell repertoire along with elevated IgE production and a graft-versus-host like systemic autoimmune disease.^{9,12} Residual recombinase activity is sufficient to generate an oligoclonal repertoire that undergoes homeostatic expansion in the immunodeficient host. The emerging principle is that oligoclonal repertoires are sufficient in effector activity but deficient in regulatory activity. Repertoires of both conventional and regulatory T cells have high levels of clonotypic diversity. However, repertoires of conventional T cells may be more robust in terms of clonal size than repertoires of regulatory T cells. A partial immunodeficiency may result in a “hole in

the regulatory T-cell repertoire” for particular self antigens without a commensurate abrogation of the conventional T-cell repertoire for the same self-antigen. The predicted outcome would be an outbreak of uncontrolled autoimmunity.

Regulatory T cells (Tregs) mediate a central role in the maintenance of peripheral self tolerance.¹³⁻¹⁵ Tregs are an important subset that typically comprises 5–10% of the CD4⁺ T-cell population. Like conventional T cells, Tregs express clonotypic, somatically-rearranged TCR and are positively-selected in the thymus upon interaction with self peptides. Mature Tregs exhibit various degrees of self-specificity and require antigenic stimulation to become suppressive. This subset exhibits an activated phenotype in normal non-stimulated animals characterized by expression of activation markers such as CD25, CTLA-4, LAG3, GITR and Foxp3. One presumes that cognate self peptides drive the activated status of Tregs in vivo. Although Tregs comprise a minority of T cells, like conventional T cells, Tregs have a highly diverse T-cell repertoire. Their overall mode of self/nonself discrimination, and particularly whether Tregs recognize self peptides in the same manner as conventional T cells, are issues that are not currently understood.

The main point is that self peptides appear critical for development of both conventional and regulatory T cell subsets. When conventional and regulatory subsets share specificity for the same self antigen, the presumption is that Tregs prevent pathogenic responses by conventional clonotypes specific for that self antigen. When focusing on a T-cell repertoire specific for a given self antigen, the probability is that a partial T-cell immunodeficiency is more likely to abrogate a minor antigen-specific T-cell network (Tregs) than a major network thereby unleashing the more predominant subset of conventional T cells against that self antigen to cause immune dysregulation and autoimmunity.⁹

Hallmarks of Normal and Pathogenic Immunity

The signature of an infectious pathogen is the sudden appearance of multiple highly

agonistic MHC-peptide ligands.¹⁶⁻¹⁸ In this sense, the immune system is a homeostatic network of weak peptide-specific interactions that is allergic to the sudden novelty of multiple strong interactions. Infectious pathogens are often characterized by rapid replication and emerge suddenly as a multitude of protein subunits. The peptides derived from infectious agents are new to the immune system and are perceived by some cross-reactive T cells as strongly efficacious agonist ligands. The diversity of agonistic recognition events most likely determines the vigor of the immune response. Pathogens giving rise to 10, 100 or 1,000s of foreign peptides will be progressively more immunogenic. Although Treg recognition of self peptides on APC dampens immune responses, Treg activity presumably is overwhelmed by threshold levels of agonistic interactions of conventional T cells with the same APC. It is tempting to think that the balance of tolerance and immunity is simply the tug of war between a highly diverse set of low efficacy interactions of Tregs with APC versus a more limited set of high efficacy interactions of conventional T cells with the same APC.

This view fits well with the idea that an interaction of molecular mimicry and mild immunodeficiency may drive autoimmune disease. Infectious pathogens evolve to mimic self molecules as camouflage against immune surveillance. Yet, mimicry is common and autoimmunity is rare. In normal immune systems, the “foreign antigen mimicry” may be recognized simultaneously by both cross-reactive repertoires of Tregs and conventional T cells. In a clonotypically-diverse immune system, a robust Treg repertoire specific for a given self antigen may cross-react extensively with the foreign mimicry, given that self and mimicry MHC-ligands must share common structural features. In this case, a robust repertoire of conventional T cells that perceive the mimicry epitope as a highly efficacious ligand would be held in check by the mimicry-specific Treg repertoire, thus preventing mimicry-specific immune pathogenesis. However, in the case of a mild or partial immunodeficiency, a cross-reactive Treg repertoire specific for the “foreign antigen mimicry” may be absent whereas the parallel, more

robust repertoire conventional T cells may be reduced but not depleted by the immunodeficiency. Thus, the minority Treg repertoire and the majority repertoire of conventional T cells may be differentially affected by the immunodeficiency; the first being abrogated and the second merely reduced in scope simply because the first is a minor subset and the second is a major subset. In this case, the residual conventional T cells may recognize the “foreign antigen mimicry” unchecked by Tregs. To whatever extent conventional clonotypes were depleted by the partial immunodeficiency, these T cells would quickly recover numbers due to recognition of the mimicry epitope in the absence of Tregs. The consequence would be clonal expansion of mimicry-specific conventional T cells, differentiation to effector status, and autoimmunity against the cross-reactive self epitope.

A Diversity of Hygiene Hypotheses

The other piece of the puzzle is the striking observation that immunological diseases almost unknown in agrarian cultures have reached epidemic levels in advanced industrial cultures. The ‘hygiene hypothesis’ was forwarded as an explanation for this paradox.¹⁹ The hypothesis is that the infectious disease load and bombardment of the innate receptor systems early in life balances the effector pathways of the developing immune system. Just as sight is needed for development of the visual cortex, exposure to microbial denizens is postulated to be critical for balanced development of the immune system. Agrarian environments are considered replete with macrophage-activating organisms which are believed to impart a Th1 bias to the developing immune system. The sterile environments of the modern industrial age lack this developmental Th1 bias and are hypothesized to foster immune systems with a pronounced Th2 bias coupled with susceptibility to Th2-associated immunological diseases such as asthma.

A variety of hygiene hypotheses have been proposed.¹⁹⁻²³ These hypotheses differ in emphasis on whether parasitic infections per se, overall infectious disease burden, innocuous bacteria of the GI tract,

engagement of the innate immune system, Th2-biased immune deviation, among other factors, may represent the key protective environmental factors. To date, the hygiene hypothesis has not been used successfully to identify surrogate agents that replicate the effect of an agrarian environment. In a broad sense, the hygiene hypothesis is confounded by a number of observations. Inner city environments have a less appealing fit of a generally poor hygienic living condition, low income and high infection rates with extremely high rates of immunological disease, in particular asthma. The role of immune deviation is uncertain. Parasites are associated with Th2 immunity but confer protection against Th2-related allergic disease. The hygiene hypothesis must not only explain increased incidence of Th2-related atopic diseases but must also account for diseases such as multiple sclerosis, inflammatory bowel disease and type I diabetes, which are thought to stem from Th1-biased autoimmune responses.

An alternative hygiene hypothesis can be formulated based on the theme of peptides being the foremost differentiation factors for development and maintenance of a balanced immune system. The emphasis has been on ‘self’ peptides driving T-cell maturation pathways. Innocuous foreign peptides however permeate our respiratory and digestive systems, and when present chronically in our environment, these peptides would have a persistent presence within our bodies. This alternative hygiene hypothesis postulates that the diversity of innocuous environmental peptides introduced constantly into our bodies is the critical variable of the environment that is of fundamental importance for development of a healthy immune system. The lack of a diverse array of innocuous foreign antigens in our contemporary ‘clean’ environments is postulated to be an important cause of immunological disease. The hypothesis is based on a number of considerations.

The developing immune system cannot distinguish origins of a particular peptide. Rather, the immune system senses whether a peptide has a transient or chronic presence, engendering immunity or tolerance respectively. In a healthy immune system, there is no immunological distinction

between self peptides and innocuous environmental peptides, if both sets have a persistent presence *in vivo*.

Foreign peptides might be considered unimportant given the vast quantitative excess of self inside the body. However, the design of the immune system imposes qualitative constraints on the size of the relevant peptide universe. Although self peptides may be in molar excess, the universe of self peptides is limited by the finite nature of the human genome and by the binding constraints imposed by the inherited set of MHC molecules. The inherited set of MHC glycoproteins acts as a filter so that only a limited subset of peptides can engage the immune system. Most peptides lack the appropriate binding motifs and are ignored by the immune system. The filtering, peptide-sampling mechanism of MHC binding represents the first step of an adaptive immune response and functions to ensure that the T-cell repertoire is not overwhelmed by essentially-infinite and extensively cross-reactive pools of self and nonself peptides.

Measured strictly in qualitative terms of peptide diversity, the universe of self peptides is dwarfed by the universe of foreign peptides. Peptide diversity can be defined as the number of different peptide species (rather than the molar concentration of any one) that are able to bind a set of inherited MHC molecules within one individual. Whereas the pool of relevant self peptides is derived from one genome, MHC-compatible foreign peptides would be derived from myriads of organisms that make up an entire ecological niche. Although self peptides may be present in molar excess *in vivo*, the universe of foreign peptides would be qualitatively more diverse, perhaps by orders of magnitude, giving rise to a much richer array of peptides with high affinity for self MHC molecules.

One might suspect that foreign peptides enter the body only in limited quantity. If so, the absorptive capacity of the mucosa is not the limiting factor. Respiratory and gut mucosal tissues are designed to maximize absorptive surface area. Depending on the physical environment, considerable quantities of aerosolized plant and animal matter may be constantly deposited in the respiratory and

gut mucosa. Enormous quantities of food and commensal organisms are present in the gut. These substances and their partially degraded products move across the mucosa via leakage and active transport. The mucosa is closely interconnected with an extensive lymphoid network comprising approximately 75% of all lymphocytes in the body. M cells actively transport antigenic material by transcytosis directly into the grasp of submucosal dendritic cells. Mucosal dendritic cells directly extend arborizations across the epithelial barrier to directly capture and internalize luminal antigenic material. By these and many other mechanisms, the mucosal immune system appears designed to maximize interactions of luminal antigenic material with submucosal leukocytes.

One of the presumptions of this hypothesis is that foreign environmental peptides have a beneficial impact on the development of T cell repertoire. However, the question arises as to how a T cell repertoire, after positive selection on exclusively self MHC ligands in the thymus, would interact with this universe of environmental peptides. Presumably, foreign peptides are absent from the thymus. The answer may lie in two hallmarks of immune system function, which are the tendency for cross-reactivity and a tendency to gravitate toward a homeostatic set-point. The T cell repertoire is selected by interactions with self MHC ligands that are perceived as weak, low efficacy ligands. These T cells exhibit cross-reactivity with foreign MHC ligands that are perceived as fully agonistic MHC ligands. Cross-reactive recognition of strong agonist peptides is necessary for immune responses against infectious agents. In all probability, mature T cells also exhibit cross-reactivity between weak self-MHC ligands and foreign MHC ligands that are also perceived as inefficient, low efficacy ligands. This latter type of cross-reactivity however would not drive an immunogenic response and is largely ignored in immunology. But this type of cross-reactivity may be important in that a substantial repertoire of T cells may have cross-reactive interactions with environmental peptides and perceive these as low efficacy or weak MHC ligands. Additional T cells

may recognize these innocuous foreign peptides as full agonists, but due to the chronic presence of these peptides from early life, this repertoire may exhibit habituation. Mechanisms of peripheral tolerance may enforce the homeostatic set-point by clonal deletion or adaptation such that these peptides, like true self peptides, become recognized as low-efficacy MHC ligands.

If environmental antigens have homeostatic interactions with mucosal T cells and are recognized as low efficacy ligands, a central question is how 'weak' recognition of foreign environmental peptides shapes T cell selection and differentiation, particularly in ways that are beneficial to the host. A clue to this answer may be provided by studies of homeostatic expansion.^{7,8} Naïve T cells adoptively transferred into an immunodeficient host expand to fill the 'available space.' Homeostatic expansion appears to be driven by a competition among clones for a limited set of MHC ligands such that the repertoire expands until a limited set of MHC-peptide ligands is saturated, thereby filling the 'available space.' Homeostatic expansion is clonotypic and is driven by recognition of some of the same self peptides responsible for positive thymic selection.

The main adaptive advantage of a diverse set of environment peptides in mucosal tissues is that these peptides would provide additional niches to diversify the T-cell repertoire. Conventional T cells that perceive environmental peptides as low efficacy ligands would have a niche favoring homeostatic maintenance of that clone. Regulatory T cells would differentiate to fill niches conferred by recognition of these persistent foreign peptides as if they were self-derived peptides. These niches would not exist and the clones would not persist in environments largely lacking diverse environmental antigens. The main point is that a diverse set of environment peptides would create developmental niches to engender a qualitatively more diverse repertoire. A repertoire maintained by both self and foreign peptides would have qualitatively higher diversity than a repertoire maintained solely on self peptides. And the clonotypic diversity of conventional and regulatory repertoires may be closely tied to the ability of the

repertoire to be a discriminating interface with the outside world.

Homeostatic niches created by the universe of environmental peptides would sustain additional clonotypes of both conventional and regulatory T cells in tissues having abundant exposure to environmental antigens, particularly mucosal tissues. Two types of regulatory T cells exist. The natural Tregs are derived during differentiation in the thymus upon interaction with self peptides. A separate regulatory subset of 'induced' Tregs acquires a suppressive Foxp3⁺ phenotype in peripheral tissues. An important subset of induced-Tregs may differentiate to fill the niches formed by presentation of persistent innocuous peptides from the environment. If so, these niches would position highly diverse clones of the 'iTreg' subset at the critical mucosal interface.

A central assumption is that a highly diverse repertoire can accurately distinguish pathogens whereas a restricted repertoire is a hallmark of immunodeficiency and is marked by error-prone decisions and immunological disease.⁹ A central postulate is that a rich array of environmental peptides, present as a constant during a lifetime (at least until recent generations), are critical for creating sufficient thresholds of T cell diversity among Treg and conventional subsets in our mucosal tissues. Particularly important would be the cultivation of highly diverse Treg repertoires specific for persistent environmental antigens. Environmental peptides, by enhancing clonotypic diversity of Treg subsets, may thereby extend immunological tolerance to the vast world of innocuous nonself in a given natural environment.

How would highly diverse mucosal repertoires recognize dangerous pathogens? Highly diverse repertoires of conventional and regulatory subsets maintained by diverse sets of both self and environmental antigens would maintain the appropriate balance of regulatory control and adaptive immunity. A diverse regulatory T-cell repertoire would prevent hypersensitivity against innocuous environmental antigens. The trigger for immunity would be the relatively sudden introduction of highly agonistic peptides from a novel source.^{16,17} Conventional T cells would drive reactivity against the source of these

agonists, while regulatory populations would prevent excessive inflammation in tissues having a predominance of self and innocuous non-self.

The hygiene hypothesis has been applied to a wide variety of immunological diseases, including Th2-associated atopic diseases as well as Th1-associated diseases such as type I diabetes, inflammatory bowel disease and multiple sclerosis. An important question pertains to how an immunological deficiency at the mucosa may prime autoimmunity against non-mucosal self antigens in the core of the body. The idea is that tolerance is local but a breach in tolerance is systemic. Self or environmental peptides that maintain tolerance have local tissue-specific distributions in the body. Presumably, regulatory subsets that recognize these peptides home to and are maintained by these peptides in local tissues and lymphatics, thereby filling the homeostatic niches generated by these peptides. In clean environments, the lack of environmental peptides may undercut an effective regulatory network and lead to smoldering inflammation and allergy in mucosal tissues against innocuous environmental antigens. Of course, these environmental peptides are no longer innocuous when these antigens drive chronic inflammation. The lack of effective regulation coupled with chronic inflammation and the periodic presence of antigenic mimicry epitopes on invading mucosal pathogens could seed immunogenic responses against cross-reactive self antigens. If molecular mimicry drives autoimmunity against cross-reactive self epitopes in a beta cell protein or a myelin protein, then the consequence might be an inflammatory response against beta cells or myelin which in turn may prime self-sustaining autoimmunity and onset of Type I diabetes or multiple sclerosis. It is provocative to think that a lack of diverse environmental peptides in mucosal tissues may indirectly lead to chronic autoimmunity against true self peptides in the core systems, organs and tissues of the body.

The key concept is the idea that the immune system has a developmental handshake with the outside world, to extend peace to the vast universe of innocuous foreign antigen as an extension of the immunological sensory apparatus, such

that these foreign antigens are given the status of 'protected self.' This strategy has a tremendous adaptive advantage because otherwise we would succumb to useless immunological wars against innocuous environmental antigens, as is the case in hypersensitivity disease.

A Glimpse into a Dirtier Tomorrow

Imagine a child growing up on a farm, a child growing up indoors in front of a computer screen or a child in an inner city environment. The farm environment would be replete with aerosolized animal and plant matter carried in quantity by wind, deposited in the lungs, dissolved in saliva and swallowed into the gut. Highly diverse constellations of environmental antigens would change from season to season but would be continuous year to year, engendering constancy of these homeostatic niches. Highly diversified repertoires of conventional and regulatory T cells would colonize the niches created by peptides from this array of aerosolized plant and animal products. Intense aerobic work in an outdoor environment would move environmental antigens into the deeper recesses of the lung to nurture local networks of diversified T cells in these critical tissues. These highly diversified repertoires of conventional and regulatory T cells would ensure optimal balance of immunogenicity and tolerance. This child would not likely develop asthma or smoldering inflammation against environmental antigens that can prime autoimmunity but would exhibit robust immunity against infectious pathogens.

The child in the sterile indoors environment of electronic computer games would have minimal exposure to what would be considered a diverse array of environmental antigens. The antigen array in this environment would be better characterized by a lack of diversity and perhaps would be punctuated by high concentrations of a particular antigen. The T-cell repertoire in lung mucosa would be maintained largely by interaction with self antigens. The differentiation of a robust Treg repertoire specific for diverse innocuous foreign antigens would be absent, because diverse foreign antigens would be lacking. In this sense, the respiratory mucosa would be

populated by a partially immunodeficient repertoire with error-prone impressions of infectious nonself. The clean indoor environments are marked by a lack of diversity compensated by the presence of uniformity. The highly diverse world of environmental antigens would be replaced with high concentrations of a limited set of antigens (dust mite, pollen, spores, pet dander, etc.). This is the perfect setting for driving immunity against these highly abundant environmental antigens, particularly if such antigens are present phasically. Their presence would be marked by abrupt appearances of highly agonistic ligands in the absence of a Treg counterbalance, thereby resulting in immunity and hypersensitivity disease.

The immune system of the child in the inner city would face the same lack of nature's diversity. Again, the environment would be characterized by uniformity in antigen make-up. The purported presence of Th1-biasing infectious agents is to no avail. Infectious agents that are present temporarily cannot prime clonotypically diverse populations of mucosal Tregs. Diversity replaced with uniformity results in deficient T cell repertoires and consequently immune dysfunction. In this sense, the hypothesis predicts that the best way to develop a food allergy is to begin a diet comprised of just one kind of food. It is interesting to consider that we cannot so easily extract ourselves from our agrarian past. The immune system may need nature's diversity to seed the full course of immunological development.

Whether this hypothesis challenges the classical concept of self/nonself discrimination is simply a matter of semantics. If one stringently defines the boundary of self and nonself as what is made within our epithelial barriers, then the answer would be yes. However, if one takes the view that the body can annex or adopt the

local array of environmental antigens as house guests, treated as one of the family, as self, then the answer is no. The hypothesis simply suggests the notion that the immune system extends an umbrella of what one would normally consider self to include both self and innocuous nonself. Associated with this idea is another interesting semantic twist on a classical concept—that type I hypersensitivities such as asthma and atopy may be considered autoimmune diseases against innocuous environmental antigens.

For those of us aghast at the epidemic of bottled water, what does the future hold? If the hygiene hypothesis is not based on infectious disease burden, innate receptor engagement, and Th1/Th2 imbalance and is instead based on nature's bountiful diversity, then aerosolized particulates from the nation's farmlands and wilderness areas may become a staple of indoor environments. Yes, in the future, we may buy both 'natural' water and 'natural' air.

Acknowledgements

The author would like to thank Dr. Mike Van Scott for helpful discussion and critical reading of the manuscript.

References

- Hogquist KA, Jameson SC, Heath WR, Howard JL, Bevan MJ, Carbone FR. T cell receptor antagonist peptides induce positive selection. *Cell* 1994; 76:17-27.
- Jameson SC, Hogquist KA, Bevan MJ. Specificity and flexibility in thymic selection. *Nature* 1994; 369:750-2.
- Jameson SC, Hogquist KA, Bevan MJ. Positive selection of thymocytes. *Annu Rev Immunol* 1995; 13:93-126.
- Alam SM, Davies GM, Lin CM, Zal T, Nasholds W, Jameson SC, et al. Qualitative and quantitative differences in T cell receptor binding of agonist and antagonist ligands. *Immunity* 1999; 10:227-37.
- Santori FR, Brown SM, Lu Y, Neubert TA, Vukmanovic S. Cutting edge: positive selection induced by a self-peptide with TCR antagonist activity. *J Immunol* 2001; 167:6092-5.
- Risueno RM, van Santen HM, Alarcon B. A conformational change senses the strength of T cell receptor-ligand interaction during thymic selection. *Proc Natl Acad Sci USA* 2006; 103:9625-30.
- Boyman O, Letourneau S, Krieg C, Sprent J. Homeostatic proliferation and survival of naive and memory T cells. *Eur J Immunol* 2009; 39:2088-94.
- Takada K, Jameson SC. Naive T cell homeostasis: from awareness of space to a sense of place. *Nat Rev Immunol* 2009; 9:823-32.
- Liston A, Enders A, Siggs OM. Unravelling the association of partial T-cell immunodeficiency and immune dysregulation. *Nat Rev Immunol* 2008; 8:545-58.
- Chen X, Jensen PE. MHC class II antigen presentation and immunological abnormalities due to deficiency of MHC class II and its associated genes. *Exp Mol Pathol* 2008; 85:40-4.
- Agarwal S, Cunningham-Rundles C. Autoimmunity in common variable immunodeficiency. *Curr Allergy Asthma Rep* 2009; 9:347-52.
- Somech R, Simon AJ, Lev A, Dalal I, Spiner Z, Goldstein I, et al. Reduced central tolerance in Omenn syndrome leads to immature self-reactive oligoclonal T cells. *J Allergy Clin Immunol* 2009; 124:793-800.
- Corthay A. How do regulatory T cells work? *Scand J Immunol* 2009; 70:326-36.
- Feuerer M, Hill JA, Mathis D, Benoist C. Foxp3⁺ regulatory T cells: differentiation, specification, subphenotypes. *Nat Immunol* 2009; 10:689-95.
- Workman CJ, Szymczak-Workman AL, Collison LW, Pillai MR, Vignali DA. The development and function of regulatory T cells. *Cell Mol Life Sci* 2009; 66:2603-22.
- Mannie MD. Immune discrimination of self and nonself: a unified theory for the induction of self tolerance among thymocytes and mature peripheral T cells. *Med Hypotheses* 1993; 40:105-12.
- Mannie MD. Immunological self/nonself discrimination: integration of self vs. nonself during cognate T cell interactions with antigen-presenting cells. *Immunol Res* 1999; 19:65-87.
- Pradeu T, Carosella ED. On the definition of a criterion of immunogenicity. *Proc Natl Acad Sci USA* 2006; 103:17858-61.
- Garn H, Renz H. Epidemiological and immunological evidence for the hygiene hypothesis. *Immunobiology* 2007; 212:441-52.
- Bach JF. Infections and autoimmune diseases. *J Autoimmun* 2005; 25:74-80.
- Romagnani S. Coming back to a missing immune deviation as the main explanatory mechanism for the hygiene hypothesis. *J Allergy Clin Immunol* 2007; 119:1511-3.
- von Mutius E. Allergies, infections and the hygiene hypothesis—the epidemiological evidence. *Immunobiology* 2007; 212:433-9.
- Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. *Science* 2002; 296:490-4.