RESEARCH: IMMUNOLOGY

Proteases, Processing, and Thymic Selection

Peter Cresswell

Foreign proteins internalized by cells are degraded into peptides. These are displayed on the cell surface bound to molecules called MHC class II (major histocompatibility complex class II). It is only in this context that the $CD4^+$ T cells of the immune system can recognize these foreign peptides. The peptide binding site of MHC class II is blocked during assembly and intracellular transport by a transmembrane glycoprotein called the invariant chain (*1*). MHC class II–invariant chain complexes are delivered into the endocytic system, where the invariant chain is degraded by endosomal and lysosomal proteases, collectively known as cathepsins. A fragment of the invariant chain, CLIP (class II–associated invariant chain peptide), remains as a place holder in the binding site until its dissociation is induced by interaction of the class II molecules with another class II–like molecule (H-2M in mice and HLA-DM in humans). The unoccupied binding site is then available for peptide fragments from degraded foreign proteins. The class II–peptide complexes so generated are then transported to the cell surface.

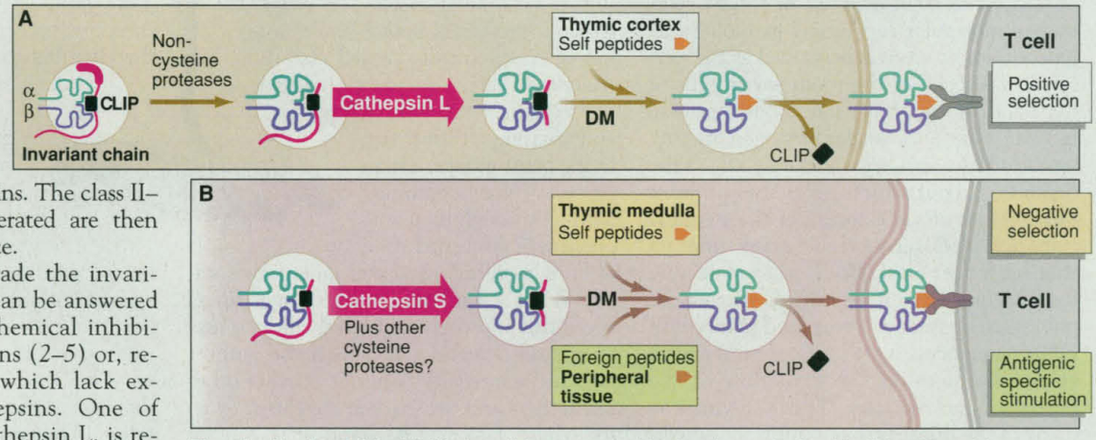
Which cathepsins degrade the invariant chain? This question can be answered with the use of specific chemical inhibitors of individual cathepsins (*2*–*5*) or, recently, "knockout" mice, which lack expression of certain cathepsins. One of these, which is missing cathepsin L, is reported by Nakagawa *et al.* on page 450 of this issue (*6*). The surprising result is that the key cathepsin or cathepsins that degrade the invariant chain and thereby generate functional class II molecules are different in different tissues.

There are two main places where the generation of MHC class II–peptide complexes is important—in the thymus and in peripheral tissues. In the thymus, developing T cells encounter MHC molecules that display peptides derived from self proteins (*7*). In the currently favored model, $CD4^+$ T cells that recognize particular class II–self peptide complexes with moderate affinity mature and escape into the periphery (positive selection). Those recognizing class II–self peptide complexes with high affinity, which might cause autoimmune disease if allowed to escape, are deleted (negative selection). $CD4^+$ T cells with receptors below a certain threshold of affinity "die by neglect" and also fail to exit the thymus. Similar processes govern the development of $CD8^+$ T cells that recognize MHC class I–peptide complexes. Positive selection is carried out by class II–positive cortical epithelial cells in the thymus, whereas negative selection is performed by medullary bone marrow–derived dendritic cells and macrophages (see the figure).

In peripheral tissues the key MHC class II–positive cell types are B cells, macrophages, and dendritic cells—collectively known as antigen-presenting cells. As well as constitutively expressing class II–self peptide complexes, these cells can express class II molecules associated with peptides derived from foreign, pathogen-derived proteins internalized during the course of an infection. These complexes are recognized in the lymph nodes or spleen by specific, mature $CD4^+$ T cells that multiply and release a variety of cytokines that amplify and modulate the overall immune response.

Cathepsin L–deficient mice exhibit a defect in the MHC class II processing pathway in thymic cortical epithelial cells. Specifically, invariant chain degradation in these cells does not proceed normally, and partial proteolytic fragments derived from it accumulate in association with class II molecules. These fragments include the CLIP region, and similar products have been previously characterized in class II–positive cells treated in vitro with inhibitors of the cysteine protease family of cathepsins (*2*–*5*). Presumably because of defective invariant chain degradation and consequent expression of a reduced repertoire of class II–peptide complexes on the cortical epithelial cells, positive selection is seriously impaired. The number of $CD4^+$ T cells in the thymus and periphery is reduced by 60 to 80%, whereas the number of $CD8^+$ cells increases to maintain the overall number of T cells. In contrast, in the bone marrow–derived medullary population responsible for negative selection, and in the peripheral class II–positive cells mediating the presentation of foreign peptides to mature $CD4^+$ T cells, the MHC class II processing pathway appears normal. No proteolytic fragments of invariant chain accumulate in spleen cells of the cathepsin L–deficient mice, and their splenocytes, dendritic cells, and macrophages are perfectly capable of generating class II–peptide complexes from a variety of cellular and internalized soluble protein antigens, stimulating specific $CD4^+$ T cells as efficiently as wild-type cells.

Cathepsin L is thus a critical protease for invariant chain degradation in thymic epithelium. But is there another enzyme that fulfills the same role in peripheral antigen-presenting



Thymic display table. MHC class II is processed in thymic cortical epithelial cells and bone marrow–derived antigen-presenting cells to display peptides. The cortical epithelial cells (**A**) mediate positive selection of $CD4^+$ T cells in the thymus and use cathepsin L for the late stages of invariant chain degradation. Bone marrow–derived antigen-presenting cells (**B**) in the thymic medulla mediate negative selection, and peripheral bone marrow–derived antigen-presenting cells present foreign peptides associated with MHC class II molecules to antigen-specific $CD4^+$ T cells. They appear to use cathepsin S for the late stages of invariant chain degradation.

The author is in the Section of Immunobiology, Howard Hughes Medical Institute Research Laboratories, Yale University School of Medicine, 310 Cedar Street, New Haven, CT 06510, USA. E-mail: peter.cresswell@yale.edu

cells? Evidence obtained by treating antigen-presenting cells in vitro with specific inhibitors suggests that cysteine proteases in general—and cathepsin S in particular—may be important in the periphery. Cathepsin S inhibition induces the accumulation of a partially proteolyzed fragment of the invariant chain in association with class II molecules and inhibits peptide loading (*4*, *5*). Nakagawa *et al.* (*6*) show that in thymus of normal mice, active cathepsin S is undetectable in cortical epithelial cells but present in thymus-derived dendritic cells and peripheral antigen-presenting cells, whereas active cathepsin L has a reciprocal distribution. Thus, the terminal stages of invariant chain degradation in thymic epithelium and in bone marrow–derived antigen-presenting cells appear to be mediated by different cathepsins.

Why should the critical invariant chain processing enzyme in thymic cortical epithelial cells differ from that in bone marrow–derived antigen-presenting cells? The range of self peptides presented to T cells undergoing negative selection in the thymus should be the same as those presented in the periphery, otherwise the potential for autoimmune recognition is high. Because the cathepsins, in addition to degrading the invariant chain, are responsible for generating the class II–associated peptides, it may be important for the proteases of the negatively selecting thymic medullary cells to be similar to those in the peripheral antigen-presenting cells. Such a restriction need not be imposed on the cortical epithelial cells mediating positive selection, where the only requirement is that a broad T cell receptor repertoire be generated. Thus, the biology of the system can be said to allow the difference in cathepsin L distribution, but the reason for it remains unclear. A further complexity is that an alternatively spliced form of the invariant chain, generally expressed together with the major form and called p41, incorporates a specific cathepsin L inhibitory domain (*8*, *9*). Does this domain regulate the activity of cathepsin L in the thymus or periphery? As is often the case, this important observation raises more questions than it answers.

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AGING

The Future of Human Longevity: A Demographer's Perspective

John R. Wilmoth

One of the greatest achievements of modern civilization has been the enormous reduction in human mortality. Life expectancy at birth, among early humans, was likely about 20 to 30 years (*1*). By 1900, the average length of life in industrialized nations had doubled relative to this historical extreme. Now, as we approach the year 2000, life expectancy at birth is around 80 years in Japan and a few other countries, and its rise continues unabated.

In recent decades, the populations of developed countries have grown considerably older, because of increasing survival to older ages as well as smaller numbers of births. Consequently, both legislators and the general public have begun to consider society's role in the support of this ever-expanding elderly population. In this new demographic context, questions about the future of human longevity have acquired a special significance for public policy and fiscal planning.

Demographers claim some expertise in predicting future mortality levels. Sometimes, their method of choice is a mere extrapolation of past trends. Biologists and others are often critical of this approach because it seems to ignore underlying mechanisms. But, in fact, this critique is valid only insofar as such mechanisms are understood with sufficient precision to offer a legitimate alternative method of prediction. Although many components of human aging and mortality have been well described, our understanding of the complex interactions of social and biological factors that determine mortality levels is still imprecise. Furthermore, even if we understood these interactions and wanted to predict future mortality on the basis of a theoretical model, we would still need to anticipate trends in each of its components.

The extrapolative approach to prediction is particularly compelling in the case of human mortality. First, mortality decline is driven by a widespread—perhaps universal—desire for a longer, healthier life. Second, historical evidence demonstrates that mortality has been falling steadily, and lifespan increasing, for more than 100 years in economically advanced societies. Third, these gains in longevity are the result of a complex array of changes (standards of living, public health, personal hygiene, medical care), with different factors playing major or minor roles in different time periods. Fourth, much of this decline can be attributed to the directed actions of individuals and institutions, whose conscious efforts to improve health and reduce mortality will continue in the future.

Predictions of future life expectancy by extrapolation yield values that are not too different from what is observed today. Recent forecasts by the U.S. Social Security Administration put life expectancy in 2050 at 77.5 years for men and 82.9 years for women, compared to 72.6 and 79.0 years in 1995 (*2*). These Social Security Administration forecasts are not true extrapolations, however, because they assume a slowdown in age-specific rates of mortality decline in the future. An independent study, based on a purely extrapolative technique, yielded more optimistic results (U.S. life expectancies at birth in 2050 of 84.3 years for both sexes combined) (*3*). Projections for Japan are only slightly higher (life expectancy at birth in 2050 of 81.3 years for men and 88.7 years for women, compared to 76.4 and 82.9 years in 1995) (*4*).

An important issue for consideration in forecasting mortality is the time frame—both the time frame of the data that form the input to an extrapolation and the time horizon of the projection itself. Although short-term fluctuations have been common, long-term mortality trends in industrialized countries have been remarkably stable. A serious yet common error is to extrapolate farther into the future than is warranted, given the length of the historical time series that forms the basis for extrapolation. When mortality decline slowed temporarily during the 1950s and '60s (in the United States and other developed countries), predictions that the rise in human life expectancy had come

The author is in the Department of Demography, University of California, Berkeley, CA 94720, USA. E-mail: jrw@demog.berkeley.edu