Systems Medicine Lecture notes Uri Alon (2020)

Lecture 7

Autoimmune disease as a fragility of surveillance against hyper-secreting mutants

[Yael Korem, Avi Mayo, Avichai Tendler, Nir Friedman and Uri Alon, Immunity, 2020]

Introduction:

Type-1 diabetes (T1D) is an auto-immune disease, in which the immune system attacks and kills beta cells. The origin of auto-immune diseases such as T1D is currently unclear – why do they exist? In this lecture we will discuss how type-1 diabetes and other common autoimmune diseases might arise from first principles. Here is the main idea: As we saw in the last lecture, the circuit that is essential for tissue size control and homeostasis has a fragility to mis-sensing mutants. To avoid mutant take-over that can be lethal, we will explore the hypothesis that the body uses the immune system to remove the mutants. These auto-immune cells thus serve an essential role, but create a fragility to auto-immune disease. Thus, there is a tradeoff between risk of autoimmune disease and risk of diseases of hyper-secreting mutant expansion. Different tissues choose among these two evils according to the evolutionary costs and benefits, providing rules for which tissues get autoimmune diseases versus mutant-expansion diseases (Fig. 7.1).

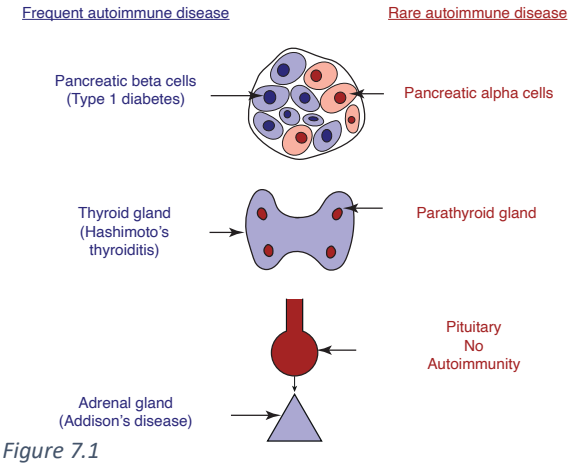

Figure 7.1

Type-1 diabetes is a disease in which the immune system kills beta-cells

In type-1 diabetes, beta-cells are attacked and killed by the body's own immune system. When enough beta-cells are killed, insulin levels in the blood are insufficient and glucose can't get into the cells from the blood. The cells starve, and switch to metabolizing fats, leading to acidification of the blood (going below the normal pH range of 7.35-7.45), which is deadly.

Thus, T1D is a lethal disease. Until the 1920s, it was a death sentence for about 1% of the world's children. Then, since the discovery of insulin by Banting and Best, T1D patients can survive and thrive by injecting insulin at the proper doses and times. But T1D still causes a lot of suffering and morbidity, and is not easy to control. It is not known how to prevent T1D, causing special concern for people at risk, such as those with a family member who has T1D. The fundamental reason that the body attacks specific cells- beta cells- is not known. As usual in medicine, when the origin is unknown, it is discussed as a combination of genetic and environmental factors. Relatively common gene variants make one susceptible (such as MHC-class-2 gene variants such as HLA-DR3 and DR4)

It is remarkable that T1D is so prevalent and has such a young age of onset (peaking around age 14), because this is a huge evolutionary cost. Natural selection should have eradicated this disease,

especially the self-killing cells. The fact that these cells are not completely eliminated raises the possibility that the disease is the dark side of an important physiological process.

Many endocrine organs have organ-specific autoimmune disease

T1D is just one of many autoimmune diseases. Autoimmune diseases are classified into systemic diseases that attack many organs (like lupus and rheumatoid arthritis), and organ-specific diseases such as T1D. Here we focus on organ-specific diseases. These diseases happen primarily in hormone-secreting organs (endocrine organs). There is a range of such disease for different endocrine organs. A very common autoimmune disease is called Hashimoto's thyroiditis (up to 5% of the population, mostly female) in which the body attacks the cells of the thyroid gland that make thyroid hormone. Killing the thyroid cells makes the person have too low thyroid hormone, and hence pathologically low metabolism. Other common diseases are Addison's disease of the adrenal cortex, vitiligo of the skin melanocytes, and gastritis of the stomach parietal cells (Fig 7.2). The origin of all of these diseases is currently unknown: they are said to be a combination of genetic and environmental factors.

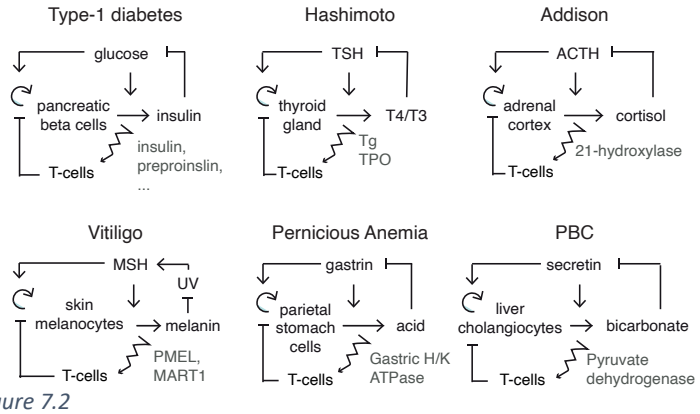

Figure 7.2

Equally puzzling is the fact that some endocrine organs virtually never get autoimmune diseases. These include the pituitary, alpha cells that secrete glucagon, parathyroid cells that secrete a hormone that controls calcium (PTH). We will try to understand why in this lecture.

All of these organ-specific diseases are due to T-cells attacking the specific cell type that secretes the hormone. Why does the immune system attack our own body cells? The immune system is designed to protect us against pathogens like bacteria and viruses, and to eliminate cancer cells. As we saw in the previous lecture T-cell, monitor the cells of the body to see if they make proteins that belong to viruses or mutated cancer proteins. T-cells that detect **self-proteins** are mostly eliminated, in, by comparison to a vast library of self in the thymus, or when activated out of context in the periphery, and by a buddy system in which each effector T cell has its inhibitory regulatory T cell.

Still, these processes do not eliminate all self-reactive T-cells: there are self-reactive T-cells in all healthy people. How these self-reactive T-cells sit quiet and what is their function is not understood (Madi et al., 2014; Semana et al., 1999; Yu et al., 2015). (Culina et al., 2018). Mainstream thought is that self-reactive T-cells are errors in the elimination mechanisms. A different line of thought in immunology is that that *self-reactive T-cells play maintenance roles in the body* [Kracht et al., 2016; schwartz and cohen 2000, Schwartz Raposo,2014]. We will go with the latter line of thought.

We explore the idea that T-cells can help to remove hyper-secreting mutants

All of the organs that get organ-specific autoimmune disease have the same regulatory motif as the beta-cells. In this feedback motif, a signal causes the cells both to secrete a hormone and to proliferate (Fig 7.2)

All of these tissues are thus sensitive to mutants that mis-sense the signal. Such mis-sensing mutants can expand and cause loss of homeostasis.

Such mutants are well known clinically. Thyroid cells with mutations in the receptor for their signal (TSH) grow into nodules that secrete too much thyroid hormone. These "toxic nodules" cause hyper-thyroidism which can be lethal. Incidentally, these nodules are not cancerous- unlike cancer, they don't give rise to new growths in other tissues called metastasis. They are instead adenomas which behave like normal thyroid cells, except that the mutant cells "think" there is too much signal. Thyroid cancer typically does not secrete thyroid hormones. Similarly, mutations in beta cells make them think there is too much glucose were described in lecture 3.

These mutants are inevitable. An organ like the thyroid weighs 10g and has 10^{10} cells. It thus takes 10^{10} cell divisions to make it. Since mutation rate is about 10^{-9}/base-pair/division, each possible point mutation will be found in about 10 thyroid cells. It is known that at least 50 such mutations cause hyper sensing and hypersecretion leading to toxic thyroid nodules. Thus every person should develop 10x50=500 toxic thyroid nodules secreting thyroid hormone- which would kill the person. Similarly, the 10^9 beta cells are sure to get enough insulin hypersecreting mutants to kill the person from hypoglycemia. Thus, just to have endocrine organs requires removal of mutants.

In lecture 3 we saw one mechanism for removing such mutants in beta cells: glucotoxicity. Glucotoxicity can cause mutants that "think" glucose is way too high to kill themselves. We noted that this mechanism still leaves the range of mild mis-sensing mutants, between the two fixed points (hatched region in Fig 7.3). Other organs, like the thyroid, do not have a mechanism like glucotoxicity at all. The signal can vary a hundred fold without causing thyroid cells to die. Thus we need another mechanism to remove mutants.

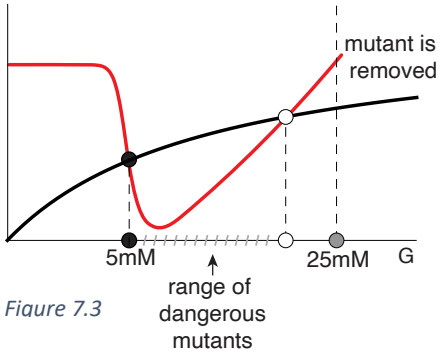

Figure 7.3

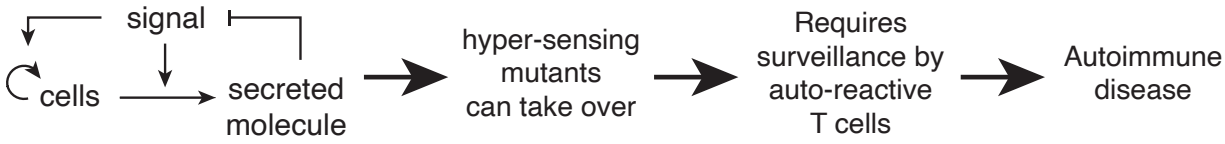

Figure 7.4

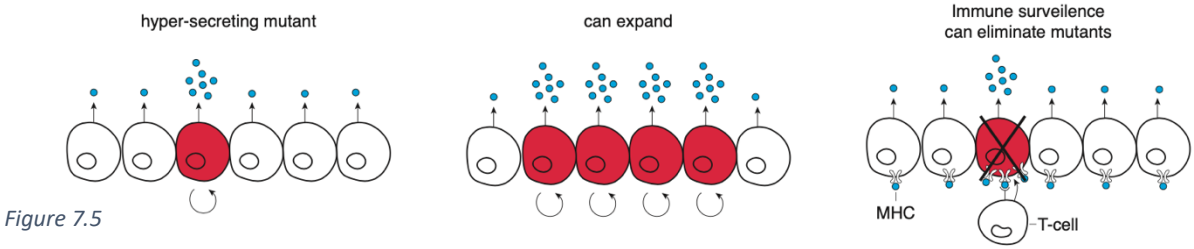

Figure 7.5

In this lecture, we will consider the idea that T-cells can help to remove mis-sensing mutants (Korem et al, 2020). To eliminate these mutants, we need a surveillance mechanism, which we will call **Autoimmune Surveillance of Hypersecreting Mutants (ASHM)** (Figs 7.4,7.5). This is a theory we developed with PhD student Yael Korem and systems immunologist Nir Friedman at Weizmann (Fig. 7.6).


Figure 7.6

ASHM requires three main features. First, it needs to detect the hyper-secreting cells. Thus, the antigens it detects must be in the secretion pathway of the hormone, made by the cells (Fig. 7.7).

Indeed, the antigens in T1D (called **auto-antigens**) are all pieces of proteins in the insulin secretion pathway. For example, a major antigen is pre-proinsulin, the protein in the cell which is cleaved to make insulin. Other major T1D auto-antigens are also proteins in the secretion pathway of insulin.

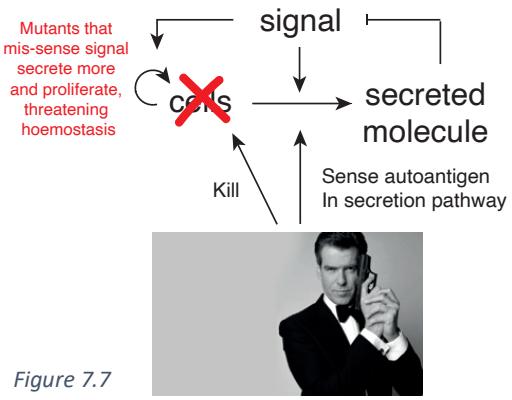

Figure 7.7

This feature is found in all of the organ-specific diseases: the auto-antigen in Hashimoto's thyroiditis is the protein cleaved to make thyroid hormone (called thyroglobulin Tg, the analog of pre-proinsulin), or the key enzyme that modifies this protein to make the hormone (TPO). In Addison's, the auto-antigen is the key enzyme that synthesizes cortisol (21- hydroxylase). Other examples are shown in Figure 7.2 and in (Table 7.1).

Autoimmune disease	Auto-antigens	Role of autoantigen
Type 1 diabetes	Insulin, preproinsulin, PTPRN, PTPRN2, islet cell antigen-69, ZnT8, GAD65	Insulin synthesis, storage and secretion
Hashimoto's thyroiditis	Thyroid peroxidase, thyroglobulin	T3/T4 biosynthesis
Addison's disease	21-hydroxylase	Cortisol/aldosterone biosynthesis
Vitiligo	PMEL, MART1, tyrosinase, tyrosinase related proteins 1 and 2	Melanin synthesis and storage
Autoimmune gastritis	Gastric H/K ATPase	Acid production
Primary Biliary Cirhosis	PDC-E2 pyruvate/oxo-glutarate dehydrogenase	Bicarbonate production

Table 7.1

Moreover, T-cells that recognize these antigens seem to be found in all healthy people. T-cells that recognize pre-proinsulin and other secretion auto-antigens are found in the T-cell repertoire shared by all people, called the **public T-cell repertoire**.

T-cell can tell the difference in antigen between neighboring cells

For immun surveillence to work, the killer T-cells need to tell which cell makes more antigen than its neighbors. In this way they can preferentially kill hyper-secreting cells. Such differential sensitivity is indeed a feature of T-cells. Experiments tested the relation between the amount of a certain antigen that a cell presents and the probability that it is killed by a T-cell that recognizes that antigen. The probability of killing is a steep function of the number of MHCs on the cell surface that present the antigen (Fig 7.8) (Martin-Blanco et al., 2018). (Halle et al., 2016).

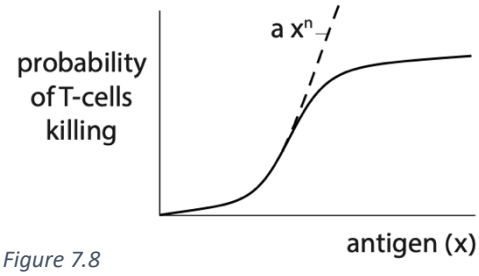

Figure 7.8

For very strong binding of antigen, a single antigen presented on a cell is probably enough. For more moderate binding, the killing rate *h(a)* goes approximately as a power law of antigen level *a*

$$(1)\ h(a) \sim c a^n\ ,$$

with a large exponent n=3-5, signifying a steep relationship. We saw such steep relationships in previous lectures, for example in glucose sensing by beta cells. Steep relationships in biology are often called '**cooperativity**', because multiple T-cell receptors in the same T-cell can cluster together and cooperate to make each other more active.

Another important property of the immune system, as we saw in the previous lecture, is that it can adapt to a background level of antigen, and only respond to temporal changes in antigen. This adaptation to background is provided by **regulatory T-cells**. As we saw, T_{regs} provide an incoherent feedforward loop circuit that has the capacity to adapt to a constant input signal (antigen level), and to respond to exponentially increasing antigen threat (Sontag, 2017). Other mechanisms exist to help the T cells adapt, such as molecular 'switches' on the T-cell that make them less active if they kill too often. The result of this adaptation is that killing rate goes according to the ratio of antigen relative to the mean antigen presented by all cells, $\mathrm{a}/<\mathrm{a}>$, so that

$$(2)\ h(a) = c\left(\frac{a}{<a>}\right)^n$$

This killing function therefore has two parameters: the rate *c* and the cooperativity *n*.

The relative sensing explains why the T-cells don't severely attack an organ if it simply starts to produce more hormone. For example when the thyroid starts producing more thyroid hormone due to a TSH-secreting tumor, or when beta cells start making more insulin due to a change in diet or insulin resistance. When more hormone is made in all of the cells of the organ, more antigen is presented, T_{regs} level rise and compensate by inhibiting the effector T-cells. The immune system thus adjusts to precisely cancel out the rise in antigen – exact adaptation we saw in the previous lecture. It remains sensitive to individual cells that make more antigen than their neighbors.

Autoimmune surveillance can eliminate any mutant, and can do so with a low killing rate

In order to work well, ASHM needs to eliminate any possible hyper-sensing mutant, and to do so without killing too many healthy beta-cells. To understand how this might work, let's analyze a mathematical model for ASHM. The main conclusion is that ASHM can eliminate mutants and kill healthy cells at a very low rate compared to their natural turnover.

We begin with the growth equation for beta-cells from lecture 2, whose growth rate (G) is controlled by glucose G:

$$(1)\ \frac{dB}{dt} = \mu(G)B$$

We will consider situations near the stable fixed-point $G = G_0 = 5mM$. Recall that the growth rate is zero at G_0, so that proliferation equals removal and beta-cell numbers are at steady-state.

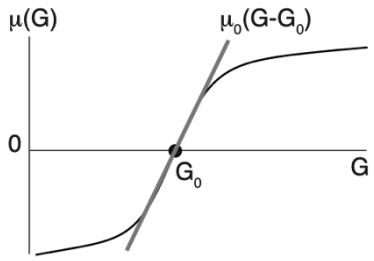

Figure 7.9

Near G_0, we can approximate the growth rate as a line with slope denoted μ_0, so that $\mu = \mu_0(G - G_0)$ (gray line in Fig 7.9). This approximation is not essential, but makes the math easier and is sufficiently accurate for our purposes. Thus

$$(2)\ \frac{dB}{dt} = \mu_0(G - G_0)B$$

Now let's add ASHM, in which beta-cells are killed by T-cells. We begin with the case in which there are only non-mutant beta-cells, called **wild-type** cells. Each cell presents *a* copies of antigen, and on average a cell presents *<a>=a* copies since we assume all cells are the same. The antigens are in the secretion pathway and hence proportional to the insulin production rate per cell, $qf(G)$. Adding the killing term from Eq. 2 to the growth equation, we find

$$(3)\frac{dB}{dt} = \mu_0(G - G_0)B - c\left(\frac{a}{<a>}\right)^n B$$

Since all cells are wild type cells, < a >=a, and the killing term is just equal to $c\ 1^n = c$. Thus, at steady state,

$$(4)\ \frac{dB}{dt} = 0 = \mu_0(G - G_0) - c$$

whose solution is the steady-state glucose level that is slightly shifted upwards from G_0 due to the effect of beta-cell killing:

$$(5)\ G_{st} = G_0 + c/\mu_0$$

The higher the killing rate *c*, the higher the glucose because more beta-cells are killed per unit time, and hence less insulin, and thus more glucose. In extreme cases, where *c* is very large, killing is widespread and we have very high glucose levels- this is the situation in T1D.

Now let's consider a mutant beta-cell that mis-senses glucose. It acts as though the true glucose level G is actually uG, where u is the **mis-sensing factor** (Fig 7.10). Such mis-sensing mutants were discussed in lecture 3. The mutants can, for example, have a mutation causing them to pump in too much glucose (James et al., 2009; Matschinsky, 2002), and as a result their insulin secretion, proliferation and removal all act as if blood glucose is higher than it actually is. In the equation for such a mutant we need to replace all occurrences of G by uG. The equation for the growth of the mutant population B_m is therefore

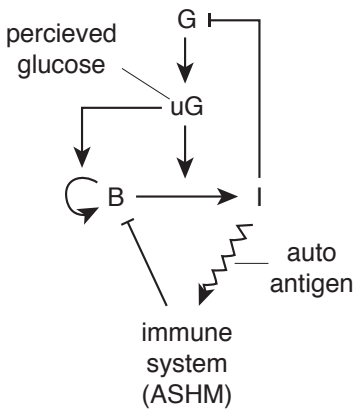

Figure 7.10

$$(6)\ \frac{dB_m}{dt} = B_m\left(\mu_0(u\,G - G_0)B - c\left(\frac{a_m}{<a>}\right)^n\right)$$

where the ASHM killing term contains the mutant antigen level a_m. Initially, there is a single mutant that arises in a wild-type tissue. Since there is only one mutant, the average antigen <a> is virtually unaffected by the mutant so that <a> can be approximated by the wild-type level of antigen. Similarly, glucose level G is not affected by the mutants as long as they are few.

The antigen level of the mutant is determined by its insulin production rate, $a_m \sim f(uG)$. Using $f(G) \sim G^2$, we find that mutant cell antigen level is $a_m = qu^2 G^2$. The wild-type antigen level is $< a >= qG^2$, because u=1 for the wild-type cells. Thus, the mutant killing term $c\left(\frac{a_m}{<a>}\right)^n$ depends only on the mis-sensing factor u, because the factors q and G^2 cancel out, leaving $(u^2)^n = cu^{2n}$. Thus:

$$(7)\ \frac{dB_m}{dt} = B_m(\mu_0(uG - G_0) - cu^{2n}) = \mu(u)B_m$$

We conclude that the mutant has a growth rate that is determined by its mis-sensing factor u:

$$(8)\ \mu(u) = \mu_0(uG - G_0) - cu^{2n}$$

In order for ASHM to work perfectly, we need the wild type cells ($u = 1$) to have the highest growth rate among all possible mutants (Fig 3.5). The wild-type cells should have zero growth rate (proliferation equal removal and thus a steady population size) and all other mutants should have negative growth rate and vanish. This is called an '**evolutionary stable strategy**' (ESS): a mechanism which cannot be invaded by any single mutant. We therefore need to find a condition such that $\mu(u)$ is maximal at $u = 1$ (Fig 7.11). This occurs when

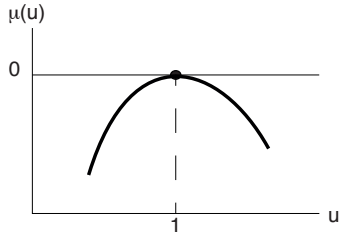

Figure 7.11

$$(9)\ \frac{d\mu(u=1)}{du} = 0 \qquad \text{condition for evolutionary stable strategy}$$

and also, that the second derivative is negative to ensure a maximum. Taking the derivative in Eq.8, we find

$$(10)\ \frac{d\mu}{du} = \mu_0 G - 2ncu^{2n-1} = 0$$

The glucose level G is just the glucose level for the wild-type case (Eq.5), because a single mutant can't affect glucose levels. Plugging in G_{st} from Eq.5, and u=1, we find the condition for ESS:

$$\frac{c}{\mu_0 G_0} = \frac{1}{2n-1}$$

This equation connects the killing rate c, normalized by the natural turnover of beta cells, to the steepness of the killing function n. Interestingly, the higher the T-cell cooperativity (steepness) parameter *n*, the lower the killing rate *c* that is required for ESS. Since the cooperativity of immune recognition is high ($n\sim$3-5), killing rate should be small, about 10-20% of the natural turnover rate $\mu_0 G_0$. Thus, these secret-agent T-cells work subtly and with high precision (Fig 7.12). Only a small part of the removals of beta-cells are due to ASHM, and the rest to natural turnover.

Figure 7.12

Surveillance can descend to autoimmune disease in several ways

This surveillance mechanism appears to work well in most people. But a small fraction of people get autoimmune disease. This process has a stochastic component- even identical twins have only about a 50% congruence in term of getting autoimmune disease.

Figure 7.13

How does the AHSM mechanism fail and descend to autoimmune disease? We don't know for sure. On theory is that a microbial infection damages the tissue, causing release of self-antigen. The infection grows exponentially and provides an inflammation danger signal. This infection may or may not have detectable symptoms. The T–cell system sees an exponentially rising amount of self-antigen in the context of inflammation. It concludes wrongly that the self-antigen, such as pre-proinsulin, is actually of viral origin.

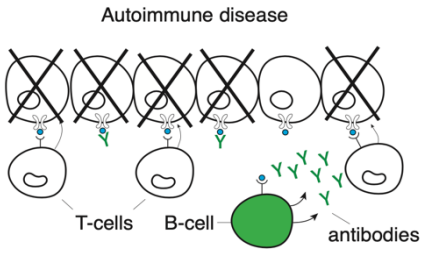

Figure 7.14

Genetic factors come into play, such as MHC variants (HLA-DR3,4) that make the MHC "platters" on antigen presenting cells present antigen more strongly. This may help to set off the heavy guns – an army of **B-cells** that produce antibodies against the antigen. The antibodies are secreted by the B-cells, and coat the beta-cells, leading a large number of immune cells to attack the beta-cells aggressively, thinking that they have virus inside them (Figs 7.13-7.14). Another possibility, raised by experiments in mouse, is that these MHC variants cause T_{regs} to undergo activation-induced cell death at high antigen levels, removing the inhibition from effector T cells and unleashing a large auto-immune response.

Note that these pro-autoimmunity genetic factors probably played a beneficial role in some past infection, perhaps by enhancing B-cell activation or reducing T_{regs} inhibition to better fight a pathogen. This tradeoff may explain why these variants are present in a sizable fraction of the population.

Normally the antibody and T-cell response would stop when the virus is eliminated and the foreign antigen is gone. In the aftermath of a viral infection, T-cells even kill each other in a process called fratricide. But in autoimmune disease, cells of the targeted tissue are attacked and killed continuously, and the immune response is not turned off. The killing releases more self-antigen, activating more immune cells, making a vicious cycle. Long lasting memory B- cells and T-cells are formed which are easily triggered by the antigen. When about 90% of the beta-cells or thyroid cells are killed, hormone production drops so low that clinical symptoms set in. A balance of beta-cell proliferation and killing

is reached in T1D patients, with very low numbers of dysfunctional beta-cells persisting for decades and a continual B-cell response (Keenan et al., 2010; Liu et al., 2009)(Rui et al., 2017).

Another possible route to autoimmunity may involve rapid growth of the tissue during puberty, coupled with some inflammation. Again, rising self-antigen levels plus alarm signals may fool the T-cells and trigger autoimmune disease, whose peak prevalence is often around puberty.

Whatever the precise route to auto-immune disease, the presence of auto-reactive T-cells provides the basic soldiers that cause a fragility to auto-immune disease.

Endocrine tissues that rarely get auto-immune disease are prone to diseases of mutant expansion

This theory predicts a tradeoff: if there is little or no surveillance in a tissue, it should get no autoimmune disease. However, it should get diseases of mutant expansion, especially at old ages after mutants have had enough time to grow into an adenoma.

We can thus look at endocrine cells and organs that very rarely have autoimmune diseases - less than 1 in $10^5 - 10^6$ lifetime prevalence. These organs include the parathyroid (PT) gland, a tiny gland that sits on top of the thyroid (Fig 7.1). Its job is to secrete the hormone PTH in order to icontrol blood calcium, which needs to be in a tight range around 1mM. PTH helps dissolve bone, which is made of calcium phosphate, in order to increase blood calcium.

The lack of autoimmune disease in this gland suggests that it has no ASHM or perhaps a very weak version. **This predicts that the gland is prone to takeover by hypersecreting mutants.** Indeed, this is a very common disease with the long name *primary hyperparathyroidism*. It afflicts about 1/50 women after menopause. A hypersecreting mutant grows exponentially and becomes a small tumor in the gland, pushing calcium levels up. The over-high calcium comes at the expense of bones, and the symptoms include loss of bone mass and neuronal problems. Treatment sometimes requires surgically removing the tumor.

The PT gland has a circuit that is sensitive to take-over by mis-sensing mutants. The circuit is essentially the same as in the other glands, except for a sign reversal. In this circuit (Fig 7.15), the signal, calcium, inhibits both the proliferation of PT-cells and secretion of PTH. Because the signs in the circuit are opposite to those of the other systems, a loss of PT-cells means low levels of calcium. A mutant that mis- senses too little calcium (hypo-sensing mutant) is the dangerous culprit: such a mutant would expand and hyper-secrete PTH, and if it takes over, lead to too high calcium.

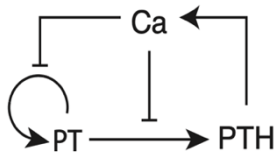

Figure 7.15

Thus, a tradeoff seems to exist between two evils: autoimmune disease and diseases of hypersecreting mutant expansion. This allows one to predict which organs will get which diseases. For example, alpha cells in the pancreas sit next to beta-cells, but unlike beta-cells, they almost never get auto-immune disease. This predicts that alpha cells should show frequent mutant expansions that hyper-secrete their hormone, glucagon, leading to excess glucose production. Such growths are indeed reported and associate with type-2 diabetes (Feng et al., 2017; Liu et al., 2011; Unger and Cherrington, 2012).

It seems that the parathyroid has at least some immune surveillance because autoimmunity in this organ can be caused by certain drugs. In particular, drug that enhance immune response against cancer, by blocking immune checkpoints, have parathyroid autoimmunity as a side effect in some patients. Thus, ASHM in this organ may be tuned to low levels that prohibit autoimmune disease under normal conditions.

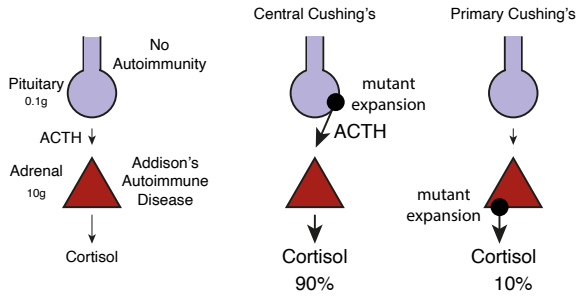

Figure 7.16

A particular clear example of the mutant/autoimmunity tradeoff seems to occur in the HPA axis. There are two glands, the pituitary and the adrenal, A and P. Each has a version of the circuit motif that is fragile to mis-sensing mutants, as we saw in lecture 4. Mutants in the pituitary that hyper-sense hormone x_1, for example, grow into nodules that hyper-secrete hormone x_2 (ACTH), making the adrenal make too much cortisol (Fig 7.16). This is known as **Cushing's syndrome**, with depression, hypertension, muscle wasting and fat distribution in the face and abdomen. An almost exact type of disorder is caused by adrenal mutants that hyper-sense x_2. However, 90% of Cushing's syndrome is caused by mutants in P, not in A. This is surprising because the number of cells in P is smaller by a factor of 100 than in A (relevant cells in the adrenal total about 10g, in the pituitary about 0.1g).

Our theory predicts then that the adrenal A is protected from mutants by ASHM, and hence should have autoimmune disease. Indeed, the adrenal A suffers from a relatively prevalent autoimmune disease called Addison's disease which destroys the adrenal by T cells. The pituitary virtually never gets such an autoimmune disease- it seems to lack ASHM. Indeed is it an immune privileged site. However, it shows relatively frequent mutant-expansion diseases- the most common form of Cushing's syndrome called central Cushing's (Fig 7.16).

Similar pituitary mutant expansion diseases plague other HP-axes. Pituitary mutant cells in the growth axis account for acromegaly and gigantism, and in other pathways to disease of hyper-gonadism and hyper-thyroidism. Again, like the adrenal, thyroid is tilted towards autoimmunity, whereas its pituitary controller cells (thyrotophs) are tilted to mutant expansion.

What rules might determine if a tissue gets autoimmune disease or diseases of mutant expansion? One possibility is based on the evolutionary cost of these diseases: it pays to set things up so that the less severe disease occurs (Fig 7.17). In beta-cells, a hypersecreting mutant expansion is lethal,

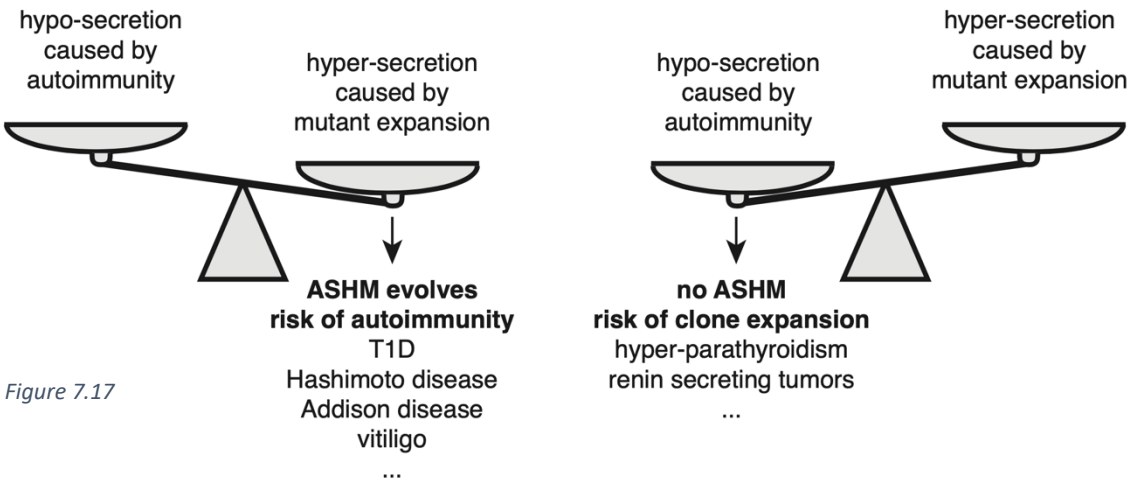

Figure 7.17

because it causes low glucose. Thus, it makes sense to sacrifice 1% of the population to T1D, to save a higher fraction of the population from lethal mutant expansion disease.

In the PT gland, in contrast, high levels of calcium caused by mutant expansion are bad but not lethal. This gland has a biphasic mechanism to protect against strong hyper-secreting mutants. The mild mutants that take over cause high calcium, but below the lethal calcium level of roughly 4 times the normal level. But even a slight reduction in calcium, as would be caused by an over active ASHM, can push calcium down to lethal levels: even a 20% reduction is lethal (below 0.9mM compared to the normal level of 1.1mM). Thus, it makes sense that ASHM does not evolve in the PT gland, to avoid the risk of low calcium. We pay the price of a less severe mutant expansion disease with a late age of onset.

But perhaps the simplest explanation for which organ gets which disease stems from the number of mutations in the organ. The smaller the organ, the less cell divisions are needed to make it, and the fewer cell divisions occur over life (assuming most glands have a similar turnover time). The fewer the mutations, the less the need for ASHM. The cutoff seems to be at a mass of about 1g, which is about 10^9 cells. Above 1g are endocrine organs with autoimmune diseases: beta cells (1g), adrenal (10g), thyroid (10g). Below 1g are glands with mutant expansion and very rare autoimmunity: pituitary cells (0.1g), parathyroid (0.3g), renin secreting cells (0.1g).

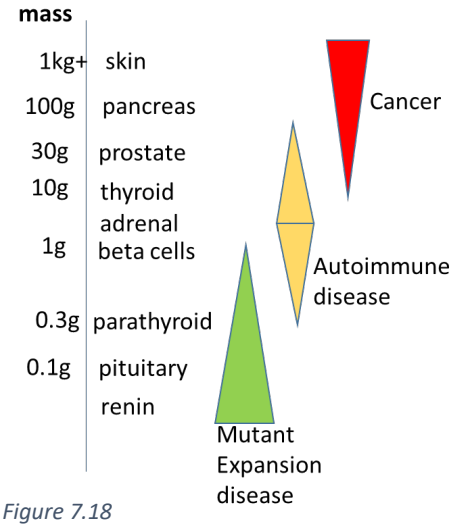

Figure 7.18

At above 10-30g, the disease spectrum shifts again, with less autoimmunity and more cancer (prostate 30g, pancreas 100g, skin several Kg). This is because there are so many cell divisions, that mutations would become too frequent. The required levels of ASHM would be too high to avoid autoimmune disease. Organs thus change strategy to stem-cell-based production, in which a single stem-cell division is amplified to make thousands of cells by transient amplifying cells. This reduces the number of mutations that remain in the stem cells of the tissue. But stem cells are more prone to cancer, being cells with high proliferation potential. In the transition zone of 10-30g one sees both cancer and autoimmunity (thyroid, prostate) (Fig. 7.18)

I like the prospect of such 'rules' for diseases, pointing towards a 'Mendeleev table' of diseases. It is fun to consider the 'diseases that might have been and are not' (such as autoimmune disease of alpha cells), and to explain them based on first principles. We will expand on this theme in the last lecture of the course.

Exercises:

7.1 **Molecular mimicry:** read about the hypothesis of 'molecular mimicry' for autoimmune diseases. Discuss its pros and cons.

7.2 **Childhood mutations:** When the embryo develops from an egg to a baby, a single cell divides to form all the cells in the body. Among these are about 10^9 beta-cells, and so there are 10^9divisions to make them.

a. Explain why in many cases there are mis-sensing mutants present already at birth.

b. Consider what happens if a mis-sensing mutation occurs early in the process of beta- cell formation, as one beta-cell divides to make two, four, eight and so on. Use this to explain why some babies are

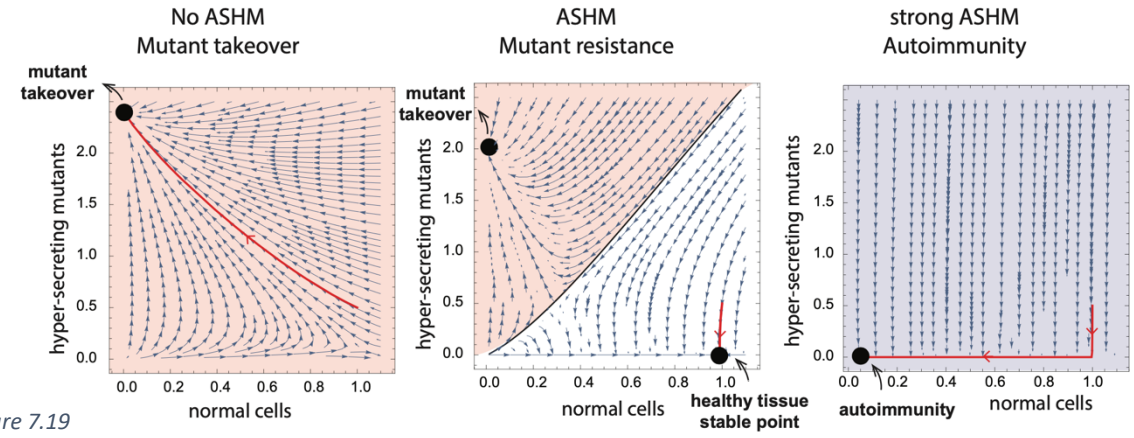

Figure 7.19

born with hyper-insulinemia (too much insulin), secreted from a part of their pancreas. Use the concept of ASHM to explain why this condition usually goes away spontaneously after a few weeks (Ackermann and Palladino, 2015).

c. In many people, the thyroid shows masses of cells that hyper-secrete thyroid hormone (called hot nodules, visible by ultrasound). These nodules usually disappear by themselves over months. Explain using the concept of ASHM.

7.3 **Route to autoimmune disease:** If a mutant manages to somehow escapes ASHM and start expanding, it can reach a size that overcomes the ASHM. This happens when average antigen <x> starts changing due to the mutant hyper-secretion. When the entire tissue is mutant, ASHM can no longer distinguish between cells, and the tissue is not attacked. Thus, ASHM is a '**frequency dependent**' mechanism, where frequency means the fraction of mutants in the cell population.

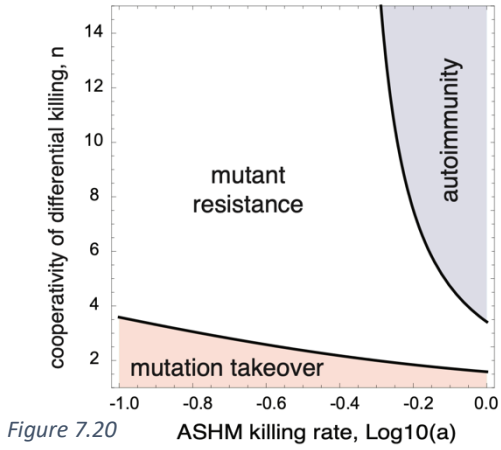

Figure 7.20

a. Estimate the number of mutants relative to wildtype cells needed to tip the balance and overcome elimination of the mutant by the ASHM.

b. Fig 7.19 shows the flow of cells in the case of a mutant with $u = 2$ competing with wild- type cells. The panels go from left to right with increasing AHSM strength parameter a. Explain the figure.

c. In rare cases, children are born a hyper-sensing mutation in their genome (germline), so that all their beta-cells have $u > 1$ and hyper secrete. This is called congenital hyper- insulinism (prevalence of about 10^{-5}). Unlike question 7.2 in which only some cells hyper-secrete, this disease does not go away on its own. It requires sometimes removing the pancreas to avoid lethally low glucose (hypoglycemia). Explain using part a, b of this question.

7.4 **range of parameters for ASHM:** Estimate the range of parameters *a* and *n* that provides protection against all mutants with $u > 2$, and kills less than 50% of the wild-type cells. Compare to Fig (7.20).

7.5 **Biphasic mechanism and ASHM:** If ASHMis good at eliminating mutant T-cells, why is the glucotoxicity mechanism also present, with its attendant risk of T2D?

7.6 **Link between two rare diseases:** Explain how ASHM might offer a link between two rare diseases. The first is polyglandular autoimmune disorder (PGAD), in which several endocrine glands show autoimmune disease in the same patient. This disorder is caused by a germ-line mutation (that is, a mutation in the fertilized egg which is present in the DNA of all cells of the body) in the thymus selection gene AIRE, responsible for eliminating self-reactive T- cells. The second disorder is called multi- endocrine neoplasm (MEN). It shows mutant expansions in multiple endocrine glands, with hormone hyper-secretion. These growths are typically non-cancerous, and are caused by germline mutations in a T-cell related gene, MEN1.

7.7 **Model for autoimmunity:** Consider the Sontag model of the previous lecture. Instead of an exponentially growing pathogen, let's assume that antigen is produced by T cells killing cells in a tissue.

(a) Explain the equation $\frac{du}{dt} = k\,T - w\,u$

(b) What is the steady state solution for u, T and R?

(c) Suppose that T_{regs}, R, are eliminated when u levels go beyond a threshold due to antigen induced cell death. An infection or damage sets of self-antigens above this threshold. What happens? Relate this to auto-immune disease. (Badami et al PNAS 2019 https://doi.org/10.1073/pnas.1910281116),

(d) In what situations of infection could the removal of R cells in part c serve a useful purpose? Discuss whether this would affect the natural selection of gene variants that produce R removal in a fraction of the population.

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