Chapter 12
Type 1 Diabetes Mellitus: Epidemiology, Genetics, Pathogenesis, and Clinical Manifestations

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Type 1 diabetes is characterized by an absolute deficiency of insulin secretion, a generally rapid onset, and dependence on exogenous insulin at the time of diagnosis. These patients are also prone to ketosis.[159]

Insulin deficiency in type 1a diabetes is caused by immune-mediated destruction of beta cells and is associated with evidence of autoimmunity. A smaller group of type 1 diabetic patients exhibit no evidence of autoimmunity and the cause of insulin deficiency remains undefined. These cases are categorized as type 1b diabetes or idiopathic type 1 diabetes and are relatively more common in African and Asian populations.[1] This category is heterogeneous, may be caused by different mechanisms in different populations, and remains poorly understood at this time. This chapter focuses on autoimmune type 1a diabetes unless otherwise specified.

Epidemiology

Type 1 diabetes is the most common form of diabetes among children and adolescents of European origin and is one of the most common chronic diseases of childhood. But this disease is not confined to childhood; cases continue to appear throughout life and approximately half the cases of type 1 diabetes are diagnosed as adults. Highlights of the descriptive epidemiology of type 1 diabetes follow.

Geographic location. One of the most striking characteristics of type 1 diabetes is the large geographic variability in the incidence of the disease (Fig. 12.1, worldwide incidence). Scandinavia and the Mediterranean island of Sardinia have the highest incidence rates in the world while oriental and equatorial populations have the lowest rates. A child in Finland is 400 times more likely to develop diabetes than one in certain regions of China. Even within Scandinavia, with genetically homogenous populations and equally developed societies living at the same latitude, incidence rates vary widely from a high of 45 per 100,000 in Finland (1996) to 28 in Sweden[2] and 20 in Denmark.[3]

The cause of this geographical variation is not immediately apparent. The existence of a strong North–South gradient and the fact that vitamin D is an immune modulator has led to speculation that decreased exposure to ultraviolet light and consequent lack of vitamin D may explain some of this gradient.[4] But there are some areas of increased incidence in sun-drenched regions (Kuwait, Puerto Rico, Sardinia) as well as some areas of very low incidence in the northern latitudes (e.g., Lithuania is only 75 miles from Finland but the incidence of diabetes is dramatically lower). So, while vitamin D levels or sun exposure may play a role, they cannot be the sole explanation of the observed variation.

Another notable feature of this geographical distribution is that great variation can be seen within the same country, even in countries with relatively homogenous populations. Thus, within China the incidence varies from 0.1 per 100,000 per year in Zunyi to 4.5 in Sichuan.[5] In Finland, the incidence is higher in the rural heartland than it is in the urban areas, though this distinction may disappear in the future because the incidence is rising faster

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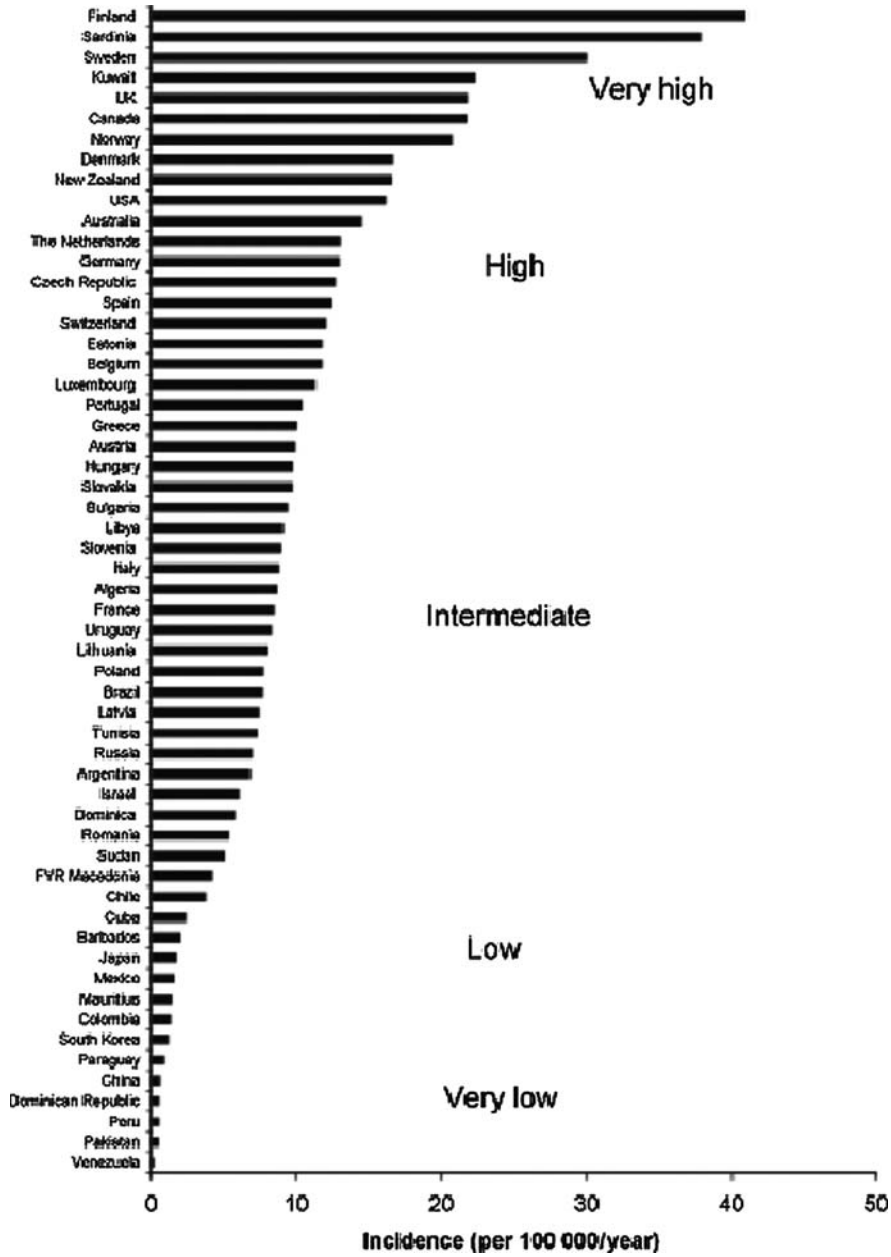

Fig. 12.1 Age-standardized incidence of type 1 diabetes in children under 14 years of age (per 100,000 per year)

in the urban population.[6] A similar trend, with higher incidence in the rural population and lower incidence in the crowded and relatively deprived urban populations, has been found in Sweden,[7] UK,[8] and Northern Ireland,[9] but not in Italy[10] or Lithuania.[11] These differences also remain unexplained at this time.

While incidence rates are much higher in European populations, the absolute number of new cases is almost equal in Asia and Europe because the population base is so much larger in Asia. It is estimated that of the 400,000 total new cases of type 1 diabetes occurring annually in all children under age 14, about half are in Asia even though the incidence rates in that continent are much lower, because the total number of children in Asia is larger.

Increase in incidence. There has been a steady increase in the incidence of type 1 diabetes in most populations studied. For example, the incidence of type 1 diabetes in Austria doubled from 7.3/100,000 in the period 1979–1984 to 14.6/100,000 in the time period 2000–2005.[12] Studies from Croatia,[13] France,[14] Germany,[15] Finland,[16] Newfoundland,[17] and China[18] all show that the incidence of type 1 diabetes is increasing at a rate of 2–5% per year. In addition, most of these studies also show that the rate of increase is greatest in the youngest subgroups (those less than 4 years of age). According to the latest report from Eurodiab,[19] countries in Eastern Europe that historically had a relatively low incidence of type 1 diabetes are the ones now showing the steepest increase. Even the Asian and African countries where the incidence was as low as 0.1/100,000 are now reporting an increase in incidence. Since the genetic composition of these populations has not changed significantly in this short time, the increase is almost certainly due to environmental factors. But in spite of intense speculation and research, the exact nature of the environmental factors that may be causing this increase remains unclear.

It should also be kept in mind that while most population groups are seeing an increase in the incidence of type 1 diabetes, this is not a universal finding. For example, at least one well-documented Swedish rural community saw no increase in the incidence of type 1 (or type 2) diabetes between 1971 and 2001[20] and the Norwegian registry did not detect any increase in incidence between 1989 and 1998.[21]

Effect of migration. In some populations migrants tend to take on the incidence rates of the host countries within one or two generations. For example, a study in Leicestershire in the UK found that type 1 diabetes incidence rates among children of South Asian origin were almost identical with those of local whites and were more than 20-fold higher than the rates reported from their ancestral homelands in South Asia.[22] This suggests that children who move from low-incidence areas to high-incidence areas can acquire the higher risk due to environmental factors. This conclusion is further supported by the observation that as type 1 diabetes has increased in incidence, the proportion of high-risk haplotypes within the diabetic population has decreased.[23] In other words, as the environment becomes more "diabetogenic," relatively lower risk haplotypes also develop diabetes and therefore the contribution of the highest risk haplotypes becomes diluted.

On the other hand, a study in Lazio (mainland Italy) found that the children of Sardinian immigrants had a type 1 diabetes incidence identical with the high incidence in Sardinia and fourfold higher than the incidence among children whose parents were native to Lazio.[24] Children with one Sardinian parent had an incidence about midway between the incidence found in Sardinia and that found in Lazio. This may indicate that in a permissive environment, migrants who carry a higher genetic risk (as Sardinians appear to do) continue to succumb at a higher rate than the rest of the population.

Age. Type 1 diabetes incidence peaks at the ages of 4–6 and 10–14 years.[25] The age distribution of type 1 diabetes onset is similar across different European populations[26] but the average age of presentation tends to be higher in African and Asian (low risk) populations. It has been suggested that these peaks coincide with higher exposure to infectious agents (at entry to school) and higher insulin demand (due to insulin resistance at puberty), but this remains to be conclusively proven. About half of all type 1 diabetics present as adults and new cases continue to present past age 70. Some adults present with evidence of autoimmunity, but with less severe insulin deficiency at presentation than is usually seen in children. These cases, which have some clinical characteristics of type 2 diabetes (relatively preserved insulin secretion, gradual onset, not dependent on exogenous insulin at diagnosis) but also exhibit evidence of autoimmunity, are sometimes said to have "Latent Autoimmune Diabetes of Adults" (LADA) and may or may not be classified as having type 1 diabetes.

Race and ethnicity. There are striking racial differences in type 1 diabetes risk in multiracial populations, although not of the same magnitude as the geographic differences. In the USA, non-Hispanic whites between the ages of 10 and 14 have an incidence rate of 32.9, which is comparable to Scandinavian populations. At the same age, the incidence rate among Hispanics is 17.6 and that in African-Americans is 19.2. Asian-Americans, with an incidence of 8.3 and American-Indians with an incidence of 7.1, have the lowest incidence rates in the American population. Comparable racial disparities have also been found in other countries. For example, in Montreal Canada, children of British descent had about 50% higher risk of type 1 diabetes than children of French descent.[27] And in a study of the incidence of diabetes mellitus in a region in Chile between 1983 and 1993, there was a significant difference between the incidence of type 1 diabetes in native Chileans (0.42/100,000) compared to Caucasian Chileans (1.58/100,000).[28] Some of the observed differences may be due to environmental factors, but others are likely to be genetic in origin. On the other hand, racially and ethnically distinct populations can

show convergence of diabetes rates (as in European and South Asian children in the UK and Arab and Jewish children in Israel[29]) and genetically similar populations can show very wide differences in diabetes incidence (for instance, the incidence in Karelians is 7.4 versus an incidence of 41 across the border in Finland[30]), indicating that environmental factors may play an even bigger role than genetic differences between populations.

Seasonality. Pooled data from many different countries show significant seasonality in date of diagnosis for type 1 diabetes in all age groups. These data show a maximum incidence in the winter period around December to January and a minimum in summer around June to July. Data from Australia and New Zealand show similar seasonality (peak incidence in winter, which in the southern hemisphere is in June and July).[31] The amplitudes of these differences are smallest for the youngest age group and largest for the oldest age group.[32,33] Very detailed and accurate records from Denmark also show that this seasonal variation seems to vary by year.[34] For example, in 2004, Denmark saw a peak during summer and it was noted that in that year summer was exceptionally wet and there was less sunshine.

On the other hand, in several populations, seasonality is absent.[35,36] It is possible that some environmental factor (for example, vitamin D, sunshine, or viral exposure) plays a role in the observed seasonality, but its effect may also be overshadowed in some populations by other genetic and environmental factors.

Another aspect of seasonality is the observation that diabetes incidence may also vary by season of birth. Thus, some studies report that the risk is higher in children born in summer (and hence, in children conceived in early winter).[37] This raises the possibility that some factor in early intrauterine life (for example, a viral infection in the mother) increases the diabetes risk in the unborn child.[38] As with so much in type 1 diabetes, this interesting hypothesis is yet to be proven.

Gender. In general, males and females have similar risk of type 1 diabetes,[39] with the pubertal peak of incidence in females preceding that in males by 1–2 years. In lower risk populations, such as Japan, there is a female preponderance with females outnumbering males by 1.4:1.[40]

Genetics of Type 1 Diabetes

While rare monogenic forms of autoimmune type 1 diabetes are known (see below), in most cases, type 1 diabetes is a complex disorder in which multiple genes and environmental factors interact to cause the disease or confer protection against it.[41]

There is a clear familial clustering of type 1 diabetes, with prevalence in siblings approaching 6% while the prevalence in the general population in the USA is only 0.4%. This difference yields a relative risk value of 15 (6/0.4). Risk of diabetes is also increased when a parent has diabetes and this risk differs between the two parents; the risk is 2% if the mother has diabetes, but 7% when the father has diabetes.[42] At this time, we have no explanation for this difference in risk transmission between fathers and mothers. Twin studies show that the heritability of type 1 diabetes is high (0.72 ± 0.21 in one population-based Danish study[43]) but is less than unity, indicating that there is also a non-shared environmental component. In monozygotic twins, the concordance rate ranges from 30 to 65%,[44] whereas dizygotic twins have a concordance rate of 6–10%. Since the concordance rate of dizygotic twins is higher than the sibling risk, factors other than the shared genotypes (for example, the shared intrauterine environment) may play a role in increasing the risk in dizygotic twins (Table 12.1).

Table 12.1 Genetic susceptibility to type 1 diabetes mellitus

European origin general population: 0.4%
Sibling: 6%
Offspring of diabetic mother: 2%
Offspring of diabetic father: 7%
Monozygotic twin: 30–65%
Dizygotic twin: 6–10%
Parents of diabetic child: 3%

It should be kept in mind that although there is a large genetic component in type 1 diabetes, 85% of newly diagnosed type 1 diabetic patients do *not* have a family member with type 1 diabetes. Thus, we cannot rely on family history to identify patients who may be at risk for the future development of type 1 diabetes as most cases will develop in individuals with no such family history.

Monogenic type 1 diabetes. Classic single-gene defects are an extremely rare cause of type 1 diabetes, but they are not unknown. In two rare syndromes (IPEX and APS-1) the genetic susceptibility that leads to diabetes is due to a classic single-gene defect. The IPEX (immune dysfunction, polyendocrinopathy, enteropathy, X-linked) syndrome is caused by mutations of the *FOXP3* gene. These mutations lead to the lack of a major population of regulatory T lymphocytes with resulting overwhelming autoimmunity and development of diabetes (as early as 2 days of age) in approximately 80% of the children with this disorder.

The APS-I syndrome (autoimmune polyendocrinopathy syndrome type 1) is caused by mutations of the *AIRE* (autoimmune regulator) gene, leading to abnormalities in expression of peripheral antigens within the thymus and/or abnormalities of negative selection in the thymus. This results in widespread autoimmunity. Approximately 18% of children with this syndrome develop type 1a diabetes.

Genes altering the risk of autoimmune type 1 diabetes. As noted above, most patients with type 1 diabetes do not have single-gene defects. Instead, their risk of developing type 1 diabetes is modified by the influence of several risk loci. The genomic region with by far the greatest contribution to the risk of type 1 diabetes is the major histocompatibility complex on chromosome 6. One other region which consistently shows up in genetic studies is the promoter region 5′ of the insulin gene on chromosome 11. More recent studies have identified several other risk loci (Fig. 12.2) but except for PTPN22, their contribution is relatively small, thus making them less useful for predicting the genetic risk of type 1 diabetes in a given individual.

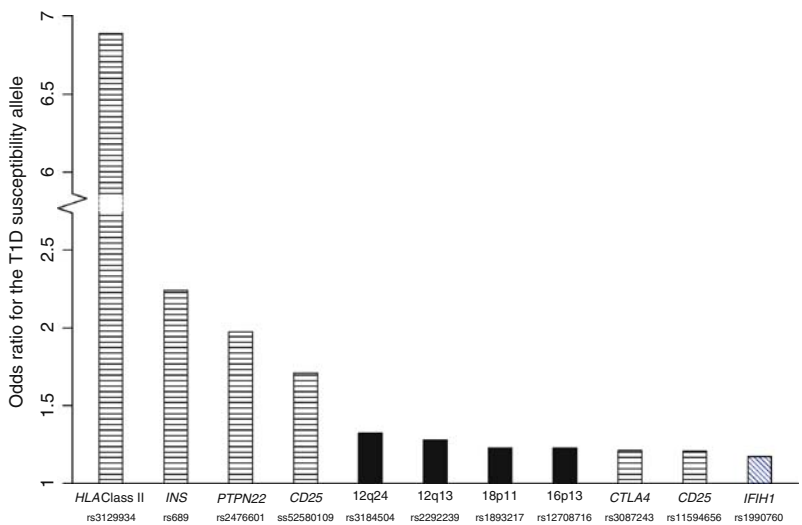

Fig. 12.2 Odds ratios for the susceptibility allele for the ten independent T1D-associated genes or regions

MHC/HLA encoded susceptibility to type 1 diabetes. The major histocompatibility complex (MHC) is a large genomic region or gene family that is found in most vertebrates and that encodes a variety of genes that are involved in immune recognition and response. In humans, the MHC region is usually referred to as the HLA (human leukocyte antigen) region and it is a superlocus that contains a large number of genes related to immune system function in humans (Fig. 12.3). These genes are further divided into HLA class I, II, III, and IV genes. Class I HLA genes encode antigens that are expressed on all body cells and include three major gene types, HLA A, B, and C. HLA class II genes encode antigens that are only expressed on certain immune cells and include HLA DP, DQ, and DR antigens. Class II genes are the ones most strongly associated with risk of type 1 diabetes, but as genetic studies become more detailed, it is becoming apparent that some of the risk associated

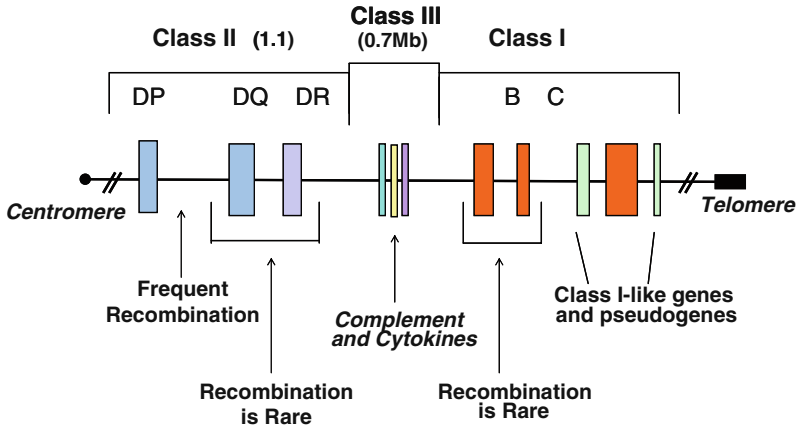

Fig. 12.3 The human leukocyte antigen complex (6p21.31)

with various HLA types is due to variation in genes in HLA classes other than class II. Overall, genetic variation in the HLA region can explain 40–50% of the genetic risk of type 1 diabetes.[45]

Initially, much of the risk associated with diabetes appeared to be linked to DR3 and DR4 alleles, but the genes of the HLA locus display strong linkage disequilibrium and it is now known that some of the earlier identified risk alleles (like DR3/DR4) confer much of their increased risk because of their linkage with other alleles in the DQ region with which they are tightly linked with relatively low recombination rates.

The HLA DR3/4-DQ2/8 is a high-risk genotype which is present in 2.3% of all newborns in Colorado, but is seen in more than 30% of children who develop diabetes. Compared to a population prevalence of type 1 diabetes of approximately 1/300, DR3/4-DQ2/8 newborns from the general population have a 1/20 genetic risk. This risk of development of type 1 diabetes is even higher when the high-risk HLA haplotypes are shared with a sibling or parent with type 1 diabetes. Thus, if one sibling has type 1 diabetes and shares the same high-risk DR3/4-DQ2/8 haplotype with another sibling, then the risk of autoimmunity in the other sibling is 50%. On the other hand, if a subject happens to have the same DR3/4-DQ2/8 haplotype in the general population, he or she has a risk of only 5% (1/20). And this risk approaches 80% when siblings share both HLA haplotypes identical by descent.[46] This is known as the "relative paradox" and points to the existence of other shared genetic risk factors (most likely in the extended HLA haplotype).

With advances in genotyping, further discrimination is now possible and we can identify more specific risk ratios for specific haplotypes. For example, the DRB1*0401-DQA1*0301 g-DQB1*0302 haplotype has an odds ratio (OR) of 8.39 while the DRB1*0401-DQA1*0301 g-DQB1*0301 has an OR of 0.35, implicating the DQB1*0302 allele as a critical susceptibility allele. Risk of diabetes is influenced by both *DRB1**04 variants and DQ alleles on DR4 haplotypes.[47] Thus there is a hierarchy of *DRB1**04 haplotypes, even with the same *DQA1**0301–*DQB1**0302 alleles, with higher risk from *DRB1**0405 (OR = 11.4), *DRB1**0401 (OR = 8.4), *DRB1**0402 (OR = 3.6), and *DRB1**0404 (OR = 1.6), while *DRB1**0403 is protective (OR = 0.27). Similarly, for *DRB1**0401, variation of *DQB1* influences risk, as haplotypes with *DQB1**0302 (OR = 8.4) are highly susceptible, while those with *DQB1**0301 (OR = 0.35) are modestly protective.

There are some dramatically protective DR–DQ haplotypes [e.g., DRB1*1501-DQA1*0102-DQB1*0602 (OR = 0.03), DRB1*1401-DQA1*0101-DQB1*0503 (OR = 0.02), and DRB1*0701-DQA1*0201-DQB1*0303 (OR = 0.02)]. The DR2 haplotype (DRB1*1501-DQA1*0102-DQB1*0602) is dominantly protective and is present in 20% of general population but is seen in only 1% of type 1A diabetes patients (Table 12.2).[48]

Role of aspartate at position 57 in DQB1. DQB1*0302 (high risk for diabetes) differs from DQB1*0301 (protective against diabetes) only at position 57, where it lacks an aspartic acid residue.[49] The DQB1*0201 allele (increased risk for diabetes) also lacks aspartic acid at position 57, and it has been proposed that this residue may be involved in the molecular mechanism underlying diabetes susceptibility.[50] It has been proposed that the presence of aspartate at this position alters the protein recognition and protein binding characteristics of this molecule.[51] But while the absence of aspartate at this position appears to be important in most Caucasian

Table 12.2 *HLA-DRB1∗04* and *DQB1* effects on type 1 diabetes risk

HLA-DRB1∗04	*HLA-DQB1*	Odds ratio
0405	0302	11.4
0401	0302	8.4
0402	0302	3.6
0404	0302	1.6
0403	0302	0.27
0401	0301	0.35

studies, it does not have the same role in Korean[52] and Japanese[53] populations. Moreover, certain low-risk DQB1 genotypes also lack aspartic acid at position 57, including DQB1*0302/DQB1*0201 (DR7) and DQB1*0201 (DR3)/DQB1*0201 (DR7). Thus the presence of aspartate at this position is usually, but not always, protective in Caucasian populations. In other populations, it may even be associated with increased risk in association with particular haplotypes.

Role of HLA class I. While the alleles of class II HLA genes appear to have the strongest associations with diabetes, recent genotyping studies and analyses of pooled data have identified associations with other elements in the HLA complex, especially HLA-A and HLA-B. The most significant association is with HLA-B39, which confers high risk for type 1A diabetes in three different populations, makes up the majority of the signal from HLA-B, and is associated with a lower age of onset of the disease.[54]

The above-mentioned HLA-risk haplotypes appear to confer increased risk in all populations, but they are not equally distributed in different populations. Part of the reason for the lower incidence of type 1 diabetes in Asian populations is lower prevalence of the highest risk haplotypes in those populations and the existence of unique haplotypes in which the high-risk alleles are associated with protective alleles.[55]

The Insulin Gene Locus, IDDM2. The second locus found to be associated with risk of type 1 diabetes was labeled IDDM2 and has been localized a region upstream of the insulin gene (5′ of the insulin gene). It is estimated that this locus accounts for about 10% of the familial risk of type 1 diabetes.[56] Susceptibility in this region has been primarily mapped to a variable number of tandem repeats (VNTR) about 500 bp upstream of the insulin gene.[57] This highly polymorphic region consists of anywhere from 30 to several hundred repeats of a 14–15 bp unit sequence (ACAGGGGTCTGGGG). The number of repeats tends to cluster into three ranges: class I (short) with 26–63 repeats, class II (intermediate) with an average of 85 repeats, and class III (long) with 140–210 repeats. Caucasians and Asians mostly have class I and class III alleles and class II alleles are relatively rare in these populations, but somewhat more common in Africans (in line with the generally greater diversity of haplotypes in the older African population).[58]

Class I (short) alleles are associated with a higher risk of type 1 diabetes, while class III (longer) alleles appear to be protective. Thus, homozygosity for class I alleles is found in 75–85% of diabetic patients, as compared to a frequency of 50–60% in the general population. It has been hypothesized that this locus alters the risk of type 1 diabetes by altering immune tolerance of insulin and this effect is due to a variation in insulin production in thymic cells, with smaller alleles being associated with lower insulin production.[59] An effect of this locus on IGF-2 transcription was also postulated, but has not been confirmed.[60]

PTPN22 (lymphoid tyrosine phosphatase). In 2004, it was reported that a single-nucleotide polymorphism (SNP) in the *PTPN22* gene on chromosome 1p13 that encodes lymphoid tyrosine phosphatase (Lyp) correlates strongly with the incidence of type 1 diabetes in two independent populations.[61] Since then, this discovery had been replicated in several populations and the gene has been found to have an association with several other autoimmune diseases.[62]

Lyp is an enzyme that has a role in signal transduction downstream of the T-cell receptor and the risk variant may represent a gain of function (increased inhibition of signal transduction), which raises the possibility that an inhibitor of this protein may hold promise as a preventive intervention in type 1 diabetes.[63]

CTLA-4. The cytotoxic T lymphocyte associated-4 (CTLA-4) gene is located on chromosome 2q33 and has been found to be associated with type 1 diabetes risk[64] as well as the risk of other autoimmune disorders[65] in

several studies. This gene is a negative regulator of T cell activation and therefore is a good biological candidate for type 1 diabetes risk modification. Because of its role in immune regulation, this gene is another candidate for therapeutic intervention and a fusion protein with human immunoglobulin is already being tested by Diabetes Trial Net as a possible preventive treatment.

IL2-receptor. SNPs in or near the gene for the interleukin-2 receptor have been found to have an association with type 1 diabetes risk.[66] Since IL2-receptor is an important modulator of immunity, it is another obvious candidate for the development of potential therapeutic interventions.

Interferon-induced helicase. Another gene that has recently been identified as having a modest effect on the risk of type 1 diabetes is the interferon-induced helicase (IFIH1) gene.[67] This gene is thought to play a role in protecting the host from viral infections and given the specificity of different helicases for different RNA viruses, it is possible that knowledge of this gene locus will help to narrow down the list of viral pathogens that may have a role in type 1 diabetes.[68]

CYP27B1. Cytochrome P450, subfamily 27, polypeptide 1 gene encodes vitamin D 1alpha hydroxylase. Because of the known role of vitamin D in immune regulation and because of epidemiologic evidence that vitamin D may play a role in type 1 diabetes, this gene was examined as a candidate gene and two SNPS were found to be associated.[69]

Other genes. Several other genes (e.g., PTPN-2) and linkage blocks, including two linkage blocks on chromosome 12 (12q13 and 12q24) and blocks on 16p13, 18p11, and 18q22 have been found to be significant in GWA studies[70,71] and further fine mapping and functional studies of genes in these regions are pending.

In addition, it has been suggested that viral infections (or other environmental factors) may activate dormant retroviruses in the human genome, or may introduce new retroviruses into the genome. A human endogenous retrovirus (IDDMK1, 222) was reported to be expressed in leukocytes from type 1 diabetes patients, but not in controls.[72] This, however, was not confirmed in subsequent studies.[73] At this time, the retroviral hypothesis remains unproven.

Environmental Factors

The fact that 50% or so of monozygotic twins are discordant for type 1 diabetes, the variation seen in urban and rural areas populated by the same ethnic group, the change in incidence that occurs with migration, the increase in incidence that has been seen in almost all populations in the last few decades, and the occurrence of seasonality all provide evidence that environmental factors also play a significant role in the causation of type 1 diabetes. The various factors that have been suggested are discussed below.

Viral infections. There are several mechanisms by which viruses may play a role in triggering or accelerating type 1 diabetes. For instance, some viruses are capable of infecting and destroying beta cells directly. In addition, viral antigens may share sequences with beta-cell antigens (molecular mimicry) or may cause the release of sequestered islet antigens (bystander damage). Repeated viral infections may induce immune dysregulation and trigger autoimmunity or aggravate pre-existing autoimmunity. Evidence for the role of several different viruses in the pathogens of type 1 diabetes is discussed below, but it should be kept in mind that most of the evidence is descriptive or suggestive, not definitive. It is possible that various viruses do play a role in the pathogenesis of type 1 diabetes, but no single virus and no single pathogenic mechanism, stands out in the environmental etiology of type 1 diabetes. Instead, a variety of viruses and mechanisms may contribute to the development of diabetes in genetically susceptible hosts.

Viruses implicated in animal models of diabetes. BBDP (*Bi*o*B*reeding *D*iabetes *P*rone) rats are prone to insulitis and type 1 diabetes and were discovered in a colony of outbred Wistar rats at the Biobreeding laboratories in Ottawa, Canada, in 1974.[74] BBDR (BioBreeding Diabetes *R*esistant) rats are derived from BBDP rats, but do not develop diabetes spontaneously. It was then discovered that if BBDR rats become infected with Kilham Rat Virus (KRV), a member of the parvovirus family, they develop type 1 diabetes. Another example in which viral infection can cause diabetes is seen in neonatal hamsters, in which rubella infection leads to diabetes.[75] The significance of these examples for humans remains unknown.

Enteroviruses. The viruses most often suspected of playing a role in type 1 diabetes are the small RNA viruses of the picornavirus family.[76] Studies have shown an increase in evidence of enteroviral infection in type 1 diabetics and an increased prevalence of enteroviral RNA in prenatal blood samples from children who subsequently developed type 1 diabetes. In addition, there are case reports[77,78] of association between enteroviral infection and subsequent type 1 diabetes. Molecular mimicry[79] and bystander damage[80] have also been suggested as mechanisms by which enteroviruses may cause type 1 diabetes. It has been proposed that some of the increase in incidence that is being seen in developed countries is due to the fact that childhood enteroviral infections have become rarer and therefore, mothers do not provide antibodies to the fetus or neonate and make them more susceptible to persistent enterovirus infection.[81] While interesting, these speculations are unproven and the true significance of enteroviral infection in type 1 diabetes remains unknown.

Congenital rubella syndrome. The clearest evidence of a role for viral infection in human type 1 diabetes is seen in congenital rubella syndrome (CRS).[82] Prenatal infection with rubella is associated with beta-cell autoimmunity in up to 70%, with development of type 1 diabetes in up to 40% of infected children. The time lag between infection and development of diabetes may be as high as 20 years. Type 1 diabetes after congenital rubella is more likely in patients that carry the higher risk genotypes. Interestingly, there appears to be no increase in risk of diabetes when rubella infection develops after birth, or when live virus rubella immunization is used. Exactly how rubella infection leads to diabetes and why it is pathogenic *only* if infection occurs prenatally, remains unknown.

Mumps virus. It has been observed that mumps infection leads to the development of beta-cell autoimmunity with high frequency, and to type 1 diabetes in some cases.[83] It has also been noted that there is an uptick in the incidence of type 1 diabetes 2–4 years after an epidemic of mumps infection.[84] But a larger European study did not find any association between mumps infection and subsequent development of diabetes. Mumps vaccination, on the other hand, appears to be protective against type 1 diabetes.[85] But while mumps may play a role in some cases of diabetes, the fact that type 1 diabetes incidence has increased steadily in several countries after universal mumps vaccination was introduced, and that incidence is extremely low in several populations where mumps is still prevalent, indicates that mumps is not an important causal factor in diabetes.

Rotavirus. Rotavirus infection in Non-Obese Diabetic (NOD) mice can involve the pancreas[86] and the rotavirus protein VP7 shows sequence homology with the autoantigens tyrosine phosphatase IA-2 and Glutamic Acid Decarboxylase (GAD).[87] But to date, there is no conclusive evidence that rotavirus infections play any role in causing or aggravating beta-cell autoimmunity in humans.

Parvoviruses. As noted above, the parvovirus KRV can induce diabetes in the BBDR rat. One case has been reported in which type 1 diabetes, Graves' disease, and rheumatoid arthritis developed in a woman after acute parvovirus infection,[88] but evidence of any large-scale association with type 1 diabetes in humans is lacking.

Cytomegalovirus (CMV). CMV viruses are capable of infecting beta cells[89] and molecular mimicry[90] is a possibility, but there is no evidence that CMV infection plays any significant role in most cases of type 1 diabetes.

Role of childhood immunizations. Several large-scale, well-designed studies have conclusively shown that routine childhood immunizations do *not* increase the risk of type 1 diabetes.[91–93] On the contrary, immunization against mumps and pertussis may decrease the risk of type 1 diabetes.[85]

The hygiene hypothesis: possible protective role of infections. While some viral infections may increase the risk of type 1 diabetes, infectious agents may also play a protective role *against* diabetes. The hygiene hypothesis states that lack of exposure to childhood infections may somehow increase an individual's chances of developing autoimmune diseases, including type 1 diabetes. Epidemiologic patterns suggest that this *may* indeed be the case. For example, rates of type 1 diabetes and other autoimmune disorders are generally lower in underdeveloped nations with high prevalence of childhood infections, and tend to increase as these countries become more developed. As noted above, the incidence of type 1 diabetes differs almost sixfold between Russian Karelia and Finland even though both are populated by a genetically related population and are located next to each other at the same latitude. The incidence of autoimmunity in the two populations varies inversely with IgE antibody levels and IgE is involved in the response to parasitic infestation. All these observations indicate that decreased exposure to certain parasites and other microbes in early childhood may lead to an increased risk of autoimmunity in later life, including autoimmune diabetes. On the other hand, retrospective case–control studies have been equivocal at best[94–96] and direct evidence of protection by childhood infections is still lacking.

In animal studies, it has been shown that diabetes can be prevented in the NOD mouse model by infecting the mice with mycobacteria, salmonella or helminthes, or even by exposing them to products of these organisms.[97–99] But the NOD mouse is not a perfect model of human type 1 diabetes and a very large number of interventions (some of them apparently trivial) can prevent the development of diabetes in this animal, so the significance of these observations for human type 1 diabetes is open to debate.

DIET. Breast feeding may lower the risk of type 1 diabetes, either directly or by delaying exposure to cow's milk protein.[100,101] Early introduction of cow's milk protein[102] and early exposure to gluten[103] have both been implicated in the development of autoimmunity and it has been suggested that this is due to the "leakiness" of the immature gut to protein antigens. Antigens that have been implicated include beta lactoglobulin,[104] a major lipocalin protein in bovine milk, which is homologous to the human protein glycodelin (PP14), a T-cell modulator. Other studies have focused on bovine serum albumin[105] as the inciting antigen, but the data are contradictory and not yet conclusive.

Other dietary factors that have been suggested at various times as playing a role in diabetes risk include omega-3 fatty acids, vitamin D, ascorbic acid, zinc, and vitamin E. Vitamin D is biologically plausible (it has a role in immune regulation), deficiency is more common in Northern countries like Finland, and there is some epidemiologic evidence that decreased vitamin D levels in pregnancy or early childhood may be associated with diabetes risk; but the evidence is not yet conclusive and it is hoped that ongoing studies like the TEDDY (the Environmental Determinants of Diabetes in the Young) study will help to resolve some of the uncertainties in this area.

Environmental chemicals. Dietary nitrosamines and nitrates can induce beta-cell autoimmunity in animal models[106] and some epidemiologic studies suggested that they may play a role in type 1 diabetes,[107] but other studies contradicted these findings and at least one large prospective study has failed to find any association with chemicals in water supply.[108] At this time, the role of environmental chemicals in type 1 diabetes awaits clarification.

Psychological stress. Several studies[109,110] show an increased prevalence of stressful psychological situations among children who subsequently developed type 1 diabetes. Whether these stresses only aggravate pre-existing autoimmunity or whether they can actually trigger autoimmunity remains unknown.

Role of insulin resistance: the accelerator hypothesis. The accelerator hypothesis proposes that type 1 and type 2 diabetes are the same disorder of insulin resistance, set against different genetic backgrounds.[111] This "strong statement" of the accelerator hypothesis has been criticized[112] as ignoring the abundant genetic and clinical evidence that the two diseases are distinct. Still, the hypothesis has focused attention on the role of insulin resistance and obesity in type 1 diabetes and there is evidence that the incidence of type 1 diabetes is indeed higher in children who exhibit more rapid weight gain.[113] Whether this is simply another factor that stresses the beta cell in the course of a primarily autoimmune disorder, or whether type 1 and type 2 diabetes can really be regarded as the same disease, is still open to question.

Pathogenesis and Natural History of Type 1 Diabetes

In type 1a diabetes mellitus, a genetically susceptible host develops autoimmunity against his or her own beta cells. What triggers this autoimmune response remains unclear at this time. In some (but *not all*) patients, this autoimmune process results in progressive destruction of beta cells until a critical mass of beta cells is lost and insulin deficiency develops. Insulin deficiency in turn leads to the onset of clinical signs and symptoms of type 1 diabetes. At the time of diagnosis, some viable beta cells are still present and these may produce enough insulin to lead to a partial remission of the disease (honeymoon period) but over time, almost all beta cells are destroyed and the patient becomes totally dependent on exogenous insulin for survival. Over time, some of these patients develop secondary complications of diabetes that appear to be related to how well controlled the diabetes has been. Thus, the natural history of type 1 diabetes involves some or all of the following stages (Fig. 12.4):

1. Initiation of autoimmunity
2. Preclinical autoimmunity with progressive loss of beta-cell function

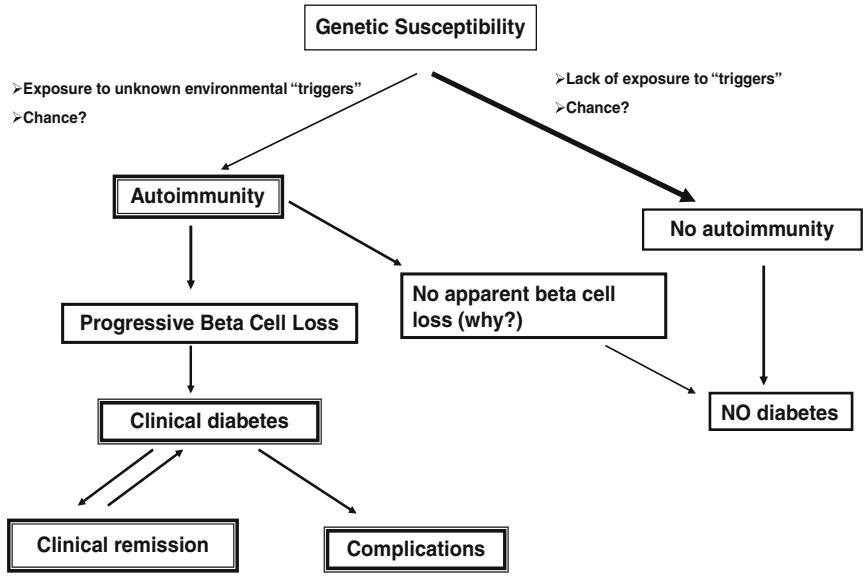

Fig. 12.4 Natural history of type 1 diabetes mellitus

3. Onset of clinical disease
4. Transient remission
5. Established disease
6. Development of complications

1. *Initiation of autoimmunity*. Genetic susceptibility to type 1 diabetes is determined by several genes (see genetics), with the largest contribution coming from variants in the HLA system. But it is important to keep in mind that even with the highest risk haplotypes, most carriers will NOT develop type 1 diabetes. Even in monozygotic twins, the concordance is 30–65%. What determines whether a genetically susceptible person goes on to develop autoimmunity is still unclear. As detailed earlier, a number of factors including prenatal influences, diet in infancy, viral infections, lack of exposure to certain infections, and even psychological stress have been implicated in the pathogenesis of type 1 diabetes, but their exact role and the mechanism by which they trigger or aggravate autoimmunity remains uncertain. What is clear is that markers of autoimmunity are much more prevalent than clinical type 1 diabetes, indicating that initiation of autoimmunity is a necessary but not a sufficient condition for type 1 diabetes.

   Whatever the triggering factor, it seems that in most cases of type 1 diabetes that are diagnosed in childhood, the onset of autoimmunity occurs very early in life. In a majority of the children diagnosed before the age of 10, the first signs of autoimmunity appear before the age of 2.[114] Development of autoimmunity is associated with the appearance of several autoantibodies. Insulin-associated antibodies (IAA) are usually the first to appear in young children, followed by glutamic acid decarboxylase 65 kDa (GAD65) and tyrosine phosphatase insulinoma-associated 2 (IA-2) antibodies. The earliest antibodies are predominantly of the IgG1 subclass. Not only is there "spreading" of autoimmunity to more antigens (IAA, then GAD 65 and IA-2) but there is also epitope spreading within one antigen. For example, initial GAD65 antibodies tend to be against the middle region or the carboxyl-terminal region, while amino-terminal antibodies usually appear later and are less common in children.[115]
2. *Preclinical autoimmunity with progressive loss of beta-cell function*. In some, but not all patients, the appearance of autoimmunity is followed by progressive destruction of beta cells. Antibodies are a marker for the presence of autoimmunity, but the actual damage to the beta cells is primarily T cell mediated.[116] Histologic analysis of the pancreas from patients with recent-onset type 1 diabetes reveals insulitis, with an infiltration of the islets of Langerhans by mononuclear cells, including T and B lymphocytes, monocytes/macrophages,

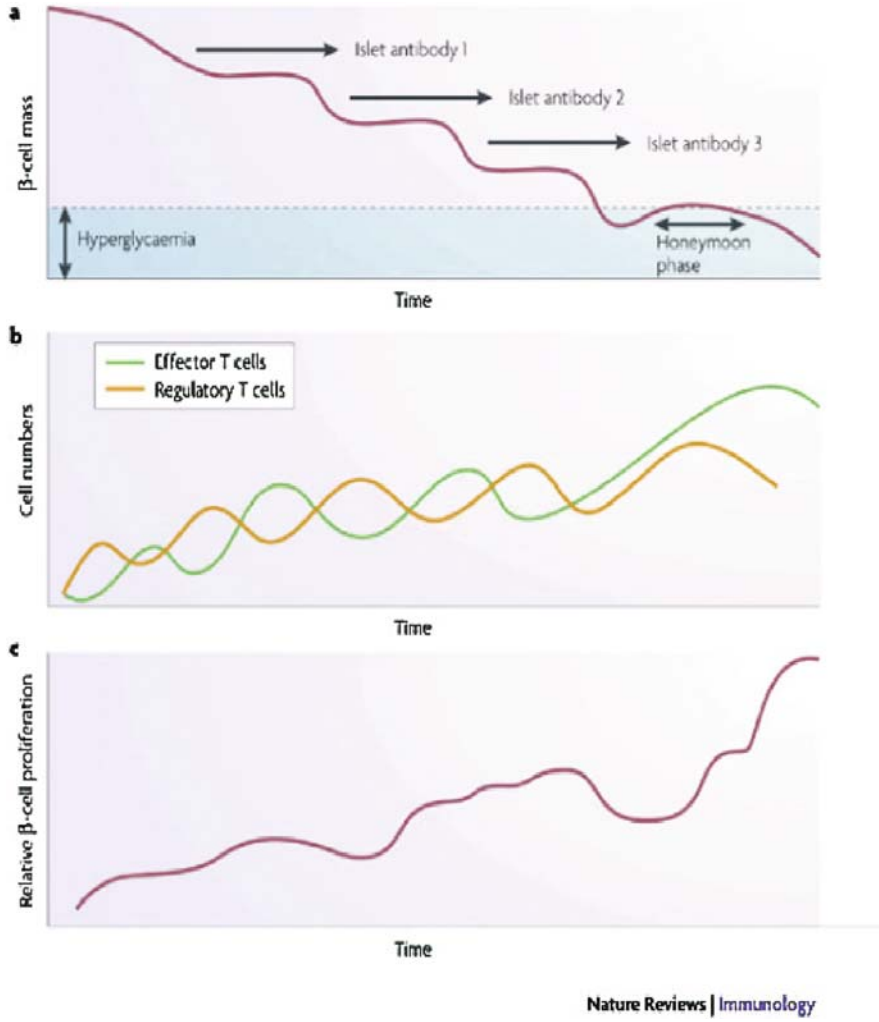

Fig. 12.5 Type 1 diabetes as a relapsing–remitting disease

and natural killer (NK) cells.[117,118] In the NOD mouse, a similar cellular infiltrate is followed by linear loss of beta cells until they completely disappear. But it appears that the process in human type 1 diabetes is not necessarily linear and there may be an undulating downhill course in the development of type 1 diabetes (Fig. 12.5).[119]

Role of autoantibodies. The risk of developing clinical disease increases dramatically with an increase in the number of antibodies; only 30% of children with one antibody will progress to diabetes, but this risk increases to 70% when two antibodies are present and 90% when three are present.[120] The risk of progression also varies with the intensity of the antibody response and those with higher antibody titers are more likely to progress to clinical disease. Another factor that appears to influence progression of beta-cell damage is the age at which autoimmunity develops; children in whom IAA antibodies appeared within the first 2 years of life rapidly developed anti-islet cell antibodies and progressed to diabetes more frequently than children in whom the first antibodies appeared between ages 5 and 8.[121]

Role of genetics in disease progression. Genetics plays a role in progression to clinical disease. In a large study of healthy children, the appearance of single antibodies is relatively common and usually transient, and does not correlate with the presence of high-risk HLA alleles,[122] but those carrying high-risk HLA alleles are

more likely to develop multiple antibodies and progress to disease. Similarly, the appearance of antibodies is more likely to predict diabetes in those with a family history of diabetes versus those with no family history of type 1 diabetes.[123] Thus, it may be the case that environmental factors can induce transient autoimmunity in many children, but those with genetic susceptibility are more likely to see progression of autoimmunity and eventual development of diabetes.

Role of environmental factors. In addition to genetic factors, environmental factors may also act as accelerators of type 1 diabetes after the initial appearance of autoimmunity. This is evident from the fact that the incidence of type 1 diabetes can vary several fold between populations that have the same prevalence of autoimmunity. For instance, the incidence of type 1 diabetes in Finland is almost fourfold higher than in Lithuania, but the incidence of autoimmunity is similar in both countries.[124]

The fact that all children with evidence of autoimmunity do not progress to diabetes indicates that there are "checkpoints" at which the autoimmune process can be halted or reversed before it progresses to full-blown diabetes. This has raised the possibility of preventing type 1 diabetes by intervening in the preclinical stage.

3. *Onset of clinical disease.* Patients with progressive beta-cell destruction will eventually present with clinical type 1 diabetes. It was thought that 90% of the total beta-cell mass is destroyed by the time clinical disease develops, but later studies have revealed that this is not always the case. It now appears that beta-cell destruction is more rapid and more complete in younger children, while in older children and adults the proportion of surviving beta cells is greater (10–20% in autopsy specimens) and some beta cells (about 1% of the normal mass) survive up to 30 years after the onset of diabetes.[125] Since autopsies are usually done on patients who died of diabetic ketoacidosis, these figures may underestimate the actual beta-cell mass present at diagnosis. Functional studies indicate that up to 40% of the insulin secretory capacity may be preserved in adults at the time of presentation of type 1 diabetes.[126] The fact that newly diagnosed diabetic individuals may still have significant surviving beta-cell mass is important because it raises the possibility of secondary prevention of type 1 diabetes. Similarly, the existence of viable beta cells, years or decades after initial presentation, indicates that even long-standing diabetic patients may be able to exhibit some recovery of beta-cell function if the autoimmune destructive process can be halted (Fig. 12.6).

Clinical features at the time of presentation range from asymptomatic (discovered on lab testing), to mild symptoms, to severe life-threatening diabetic ketoacidosis.

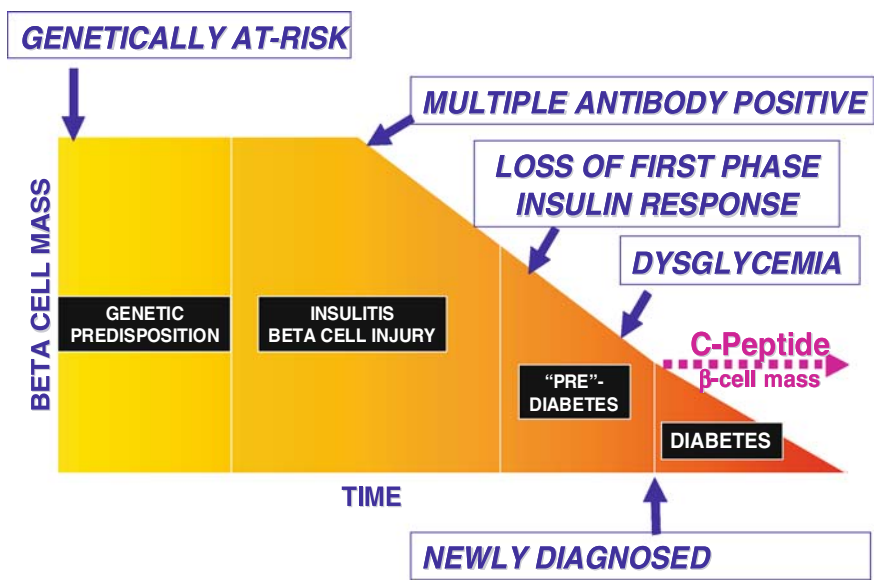

Fig. 12.6 Beta-cell mass at various stages in the natural history of diabetes

A. *Asymptomatic at diagnosis.* A small number of patients with type 1 diabetes are diagnosed before the appearance of any clinical symptoms because blood or urine testing is performed due to an unrelated illness, or in the course of a research study, or by parents who already have one diabetic child and happen to test a sibling. Such patients may need little or no treatment at diagnosis and may exhibit a prolonged "honeymoon period," but eventually almost all of them will progress to more typical type 1 diabetes.

B. *Classic presentation.* The classic presentation of type 1 diabetes is with polyuria, polydipsia, polyphagia, and weight loss.[127] With progressive loss of insulin secretion, fasting and postprandial glucose values become elevated. As blood glucose level rises, it exceeds the renal threshold for glucose (generally around 180 mg/dl) and the patient develops glucosuria. Osmotic diuresis then leads to polyuria and dehydration and this stimulates thirst, leading to polydipsia. At the same time, insulin deficiency leads to a switch from anabolic to catabolic metabolism and this, in combination with glucosuria, leads to weight loss in spite of polyphagia. Nocturnal enuresis due to polyuria is also a very common symptom in children. Other symptoms like fatigue, blurred vision, and muscle cramps may also be seen. Pyogenic skin infection and candidal vaginitis in prepubertal girls, or balanitis in uncircumcised boys, may be the presenting complaint in some cases, but careful history taking will almost invariably reveal that polyuria, polydipsia, or weight loss are also present.

C. *Diabetic ketoacidosis.* Of children with type 1 diabetes, 20–40% present with diabetic ketoacidosis (DKA). Younger patients are more likely to present with DKA, as are patients of lower socioeconomic status, female gender, and lack of family history of type 1 diabetes. Areas with a low prevalence of type 1 diabetes are also more likely to see DKA on presentation as caretakers and medical personnel are unfamiliar with the early symptoms of the disease.[128] Young children are more likely to present with DKA because younger children have lost more of their beta-cell mass at diagnosis and are more likely to have absolute insulin deficiency, and because early symptoms may be missed more frequently in very young children. Incidence of diabetic ketoacidosis at diagnosis has declined in some countries as the general public and medical professionals have become more familiar with early signs and symptoms.[129]

   Patients who present with DKA have usually had a period of polyuria and polydipsia that was not recognized as significant. The occurrence of DKA may be precipitated by a stressful event (for example, an acute infection) or may simply reflect the progression of earlier symptoms to the point that homeostatic mechanisms fail and DKA develops. As the patient becomes increasingly dehydrated and lipolysis accelerates due to lack of insulin, increased delivery of fatty acids to the liver and subsequent increase in ketogenesis develop. Increasing ketonemia leads to acidosis, which may be worsened by lactic acidosis due to dehydration. Dehydration also leads to decreased renal function, further compromising acid excretion and worsening acidosis. Acidosis may lead to CNS depression and Kussmaul respirations. Elevated ketones can also cause nausea, abdominal pain, and vomiting. An elevated leukocyte count and nonspecific elevation of serum amylase are frequently seen, but serum lipase is usually not elevated.

   The occurrence of cerebral edema may complicate 0.5–1% of cases of DKA. Mortality in patients with DKA ranges from 0.15 to 0.5% in advanced countries and 57–87% of deaths are thought to be due to cerebral edema. Other relatively rare causes of death in DKA include hypokalemia, hyperkalemia, hypoglycemia, thrombosis, septicemia, and multi-organ failure.[130] These complications and their management are discussed in Chapter 18.

D. *Acute fulminant diabetes.* An unusual form of type 1 diabetes characterized by very short history of symptoms (only a few days rather than weeks or months), rapid deterioration, minimal elevation of hemoglobin A1c in spite of severe hyperglycemia (indicating that the pathologic process is of short duration), and frequent history of recent acute illness was initially reported from Japan and Korea,[131,132] It has now been reported in at least three Caucasian adults in France as well. Typical autoimmune type 1 diabetes develops relatively slowly (over months or years) and evidence of autoimmunity is present long before the onset of clinical diabetes. In contrast, acute fulminant diabetes appears to develop in a matter of days in previously euglycemic individuals and is frequently accompanied by signs of exocrine pancreatic damage (acute pancreatitis). The occurrence of recent acute illness and evidence of viral infection are frequently seen. Evidence of autoimmunity may be seen, but the most commonly associated antibodies are directed

against amylase rather than against beta-cell antigens.[133] All these facts indicate that acute fulminant diabetes may be the result of acute pancreatitis (including autoimmune pancreatitis) and represents a disease distinct from typical type 1 diabetes.

4. *Transient remission (honeymoon period).* After initial diagnosis of type 1 diabetes, most patients experience a transient decrease in their requirements of exogenous insulin, with a small minority (2–12%) showing total remission for a variable period of time.[134] It is likely that prolonged hyperglycemia and fatty acid excess inhibits the function of otherwise viable beta cells ("glucotoxicity" and "lipotoxicity") and when normoglycemia is reestablished after diagnosis, these cells recover function and thus increase the patient's capacity to secrete endogenous insulin. Unfortunately, this natural remission is almost always temporary and insulin requirements tend to increase gradually or abruptly within a few months in most patients. In extremely rare cases, the remission may last for years.[135] Younger children tend to have a shorter remission as beta-cell destruction is more rapid and more complete in this age group.[136] Less severe initial presentation is associated with longer remission, as are low islet cell antibody and IA-2 antibody levels. Efforts to prolong or accentuate this remission are the basis for various interventions that may be regarded as secondary prevention of diabetes.
5. *Established disease.* In most patients, almost all residual beta-cell function is lost within 1–3 years of diagnosis and the patient is then totally dependent on the administration of exogenous insulin. Management of this stage is discussed in detail in Chapter 43.
6. *Chronic complications of type 1 diabetes*. Patients with type 1 diabetes develop vascular complications (microangiopathy and atherosclerosis) that can lead to cardiovascular disease, retinopathy, nephropathy, neuropathy, peripheral circulatory disease, and other forms of end-organ damage. Poor glycemic control is associated with more rapid development of complications, probably via multiple mechanisms. These are discussed in greater detail in Part VI but a few salient features are highlighted here.

    1. Cardiovascular mortality is very significantly elevated in type 1 diabetes and is 2–20-fold higher in young adults with type 1 diabetes as compared to their peers. In fact, cardiovascular disease has now overtaken nephropathy as a cause of premature death in adults with diabetes.[137–139]
    2. Atherosclerosis begins at an early stage in the disease,[140] therefore all patients with type 1 diabetes should be screened for cardiovascular risk factors like lipid levels and hypertension, and these should be aggressively treated in order to prevent premature cardiovascular disease.[141]

Associated autoimmune disorders. Autoimmune type 1 diabetes is associated with an increased incidence of several other autoimmune disorders, the most prominent of which are celiac disease and autoimmune thyroiditis. The prevalence of thyroid antibodies in children with type 1 diabetes ranges from 7 to 40% in different studies[142–144], while the prevalence of celiac disease ranges from 1 to 16.4%.[145–147] In a recent large study from Germany and Austria, the prevalence of celiac-associated antibodies was approximately 11%, while the prevalence of antithyroid antibodies was 15%.[148]

Primary prevention of type 1 diabetes. While some genetic factors clearly increase the risk of type 1 diabetes, not all high-risk subjects develop autoimmunity, and not all those who develop markers of autoimmunity go on to develop type 1 diabetes. This indicates that there are "checkpoints" on the road to diabetes at which the autoimmune process may be stopped or reversed. Intervening to prevent progression to type 1 diabetes (primary prevention) may therefore be feasible and several trials have attempted to test various interventions in this regard.

A safe, effective, inexpensive, and easily administered intervention could theoretically be targeted at all newborns, but no such universally effective intervention is yet available. Delaying the introduction of cow's milk protein, delaying introduction of cereals, and increasing the duration of breast feeding are all potentially beneficial and trials of these interventions are ongoing.[149,150] But the fact that the disease has continued to increase in incidence in Northern Europe in spite of increase in breast feeding indicates that these interventions may not be sufficient to reverse the epidemic.

Other dietary interventions that are being tested, or may be tested in high-risk subjects, include omega-3 fatty acid supplementation, vitamin D supplementation, and the use of cod liver oil during pregnancy.[151] In all these cases, there are some hints of possible benefit but nothing has been conclusively proven until now.

In high-risk populations (relatives of type 1 diabetic individuals, especially those with high-risk genotypes), it is feasible to test more targeted interventions. One of the first interventions to be tested in a high-risk population was the use of nicotinamide supplementation, but this failed to prevent type 1 diabetes.[152] Parenteral insulin[153] and nasal insulin[154] proved similarly ineffective in preventing diabetes, but oral insulin appeared to delay the incidence of diabetes in some patients.[155] A larger trial of oral insulin is currently ongoing and results are awaited.

Other studies that are ongoing or planned will look at the effect of GAD-alum and anti-CD3 antibodies in subjects at high risk for the development of type 1 diabetes.

Secondary prevention. Depending on age, anywhere from 10–20 to 40% (or more) of a person's beta cells may be intact at the time of diagnosis. In addition, small numbers of beta cells may survive (or develop anew) up to 30 years after diagnosis. This raises the possibility that diabetes can be cured or ameliorated by stopping the autoimmune destructive process *after* initial diagnosis (secondary prevention).

Immunosuppressants like cyclosporine have been tested for this purpose,[156] but while they may prolong the honeymoon period, they are associated with significant side effects and are only effective as long as they are being administered, so their use for this purpose has been abandoned. Trials using CD3 antibodies have been more promising, but some patients did develop flu-like symptoms and reactivation of Epstein–Barr Virus infection.[157] Further trials of this therapy and other therapies targeted at various components of T cells and B cells are planned or ongoing.[158]

The possibility of using glucagon-like peptide (GLP-1) agonists (e.g., exenatide) alone or in combination with immunomodulatory therapies is also being explored as these agents are capable of increasing beta-cell mass in animals (though not necessarily in humans).

Summary

Type 1 diabetes is a heterogenous clinical syndrome, characterized by absolute insulin deficiency. It can present at any age, with about half the cases being diagnosed in childhood. Most cases in children are associated with autoimmune destruction of the pancreatic beta cells. Several genes, especially certain HLA haplotypes, are associated with increased risk of the disease, but environmental factors also play a significant role and their role may be greater in older patients. It is hoped that better understanding of the disease process will lead to more accurate identification of susceptible persons and effective interventions to prevent the disease in susceptible hosts.

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