Hironori Nakagami *Editor*

Therapeutic Vaccines as Novel Immunotherapy

Biological and Clinical Concepts

Springer

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Editor
Hironori Nakagami
Department of Health Development and Medicine
Osaka University, Graduate School of Medicine
Suita, Osaka, Japan

ISBN 978-981-32-9627-5 ISBN 978-981-32-9628-2 (eBook)
https://doi.org/10.1007/978-981-32-9628-2

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The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

The first history of vaccine dates back to when Dr. Edward Jenner, who lived in the United Kingdom, contrived a way to inoculate vaccinia in prevention of smallpox at the end of the eighteenth century.

When people were infected with both a bacteria and a virus, they did not show any symptoms or have an illness at the second time of infection. Although it was well-known more than before, vaccine as a therapeutic technology started about 200 years ago. Since then, vaccine has greatly contributed to the field of medicine by providing "group immunity" which protects the population from infection, thus leading to a decline in death rates and an increase in life-span.

Recent progress of immunology allows us to understand an in-depth mechanism of this medical technology. Based on vaccine research studies, the selection of antigen and adjuvants, formulation, and delivery (intradermal, intramuscular, or transnasal) are modified to improve safety and validity.

The first history of antibody medicine treatment dates back to the nineteenth century when Dr. Kitazato Shibasaburo in Japan developed an antiserum to prevent tetanus. As you know, the antibody medicine is developing big as a trump to an incurable disease (i.e., rheumatoid arthritis) or cancer in recent years, because the antibody for specific target can directly inhibit the progression of cancer or rheumatoid arthritis, etc. We believe that vaccine not only can prevent infection but can also develop into therapy for lifestyle-related diseases or chronic diseases. Hence, the applications of vaccine have recently been expanded to treat conditions such as cancer, rheumatoid arthritis, and Alzheimer's disease, by targeting self-antigens. In the future, antibody medicine and vaccine as an immunotherapy will contribute to the increase of a healthy life expectancy.

Suita, Osaka, Japan — Hironori Nakagami

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Chapter 1
Overview: New Concept of Therapeutic Vaccines

Hironori Nakagami and Ryuichi Morishita

Abstract Recent research on vaccination has extended its scope from infectious diseases to chronic diseases from bench to patients. Vaccination against amyloid beta for amyloid plaques or phosphorylated tau for neurofibrillary tangles has been developed for the patients with Alzheimer's disease. Unfortunately, the initial promising vaccines for amyloid beta were halted during clinical trials because of adverse effects like meningoencephalitis. Based on these results, the recent vaccine will pay more attention to avoid the cytotoxic effect of immunoreaction-induced vaccine. The therapeutic vaccine mainly induces the antibody production without cytotoxic effect. Thus, in the formulation of therapeutic vaccine, the antigen excludes MHC class I and II arrangement from antigen sequence and the carrier protein is required instead. In addition, co-treatment of adjuvant is also required to break down the peripheral immune tolerance. The devise of therapeutic vaccine allows us to induce the specific antibody (efficiency) without cytotoxic T-cell reaction to target protein (safety). The therapeutic vaccine for chronic diseases might be a new optional tool in future.

Keywords Vaccine · Innate immunity · Adjuvants · T-cells · Antibody

H. Nakagami (✉)
Department of Health Development and Medicine, Osaka University, Graduate School of Medicine, Suita, Japan
e-mail: nakagami@gts.med.osaka-u.ac.jp

R. Morishita
Department of Clinical Gene Therapy, Osaka University Graduate School of Medicine, Suita, Japan

H. Nakagami (ed.), *Therapeutic Vaccines as Novel Immunotherapy*,
https://doi.org/10.1007/978-981-32-9628-2_1

1.1 Introduction

A vaccine is the remedy utilized from the old days as prevention treatment to an infection, but the spadework and the clinical test which apply this treatment technology to disease treatment of Alzheimer's disease and high blood pressure have recently started (Bachmann and Whitehead 2013; Morgan et al. 2000; Schenk et al. 1999, 2002; Tissot et al. 2008). Since lifestyle-related disease is frequent diseases in recent aged society, if it is possible to reduce medicine of treatment even a little for life by the prevention or early treatment intervention, you can contribute to reduction of medical expenses big. In addition, the therapeutic vaccine is also expected as alternative therapy of an antibody medicine (Semerano et al. 2012; Delavallée et al. 2010; Durez et al. 2014). We will get in touch with the development of a therapeutic vaccine to the patient who is continuing and is doing antibody treatment for a disease.

In this chapter, we will introduce a part of new concept in therapeutic vaccines, and the devise of immunotherapy for an endogenous protein.

1.2 Immunotherapy Based on Innate and Acquired Immunity

Immunoresponse is roughly divided into innate immunity and acquired immunity. Recent evidence demonstrated that the innate immunity, which nonspecifically reacts to the invaders (i.e., virus or bacteria), is very important to the acquired immunity which is the peculiar immunoresponse to these foreign pathogens (virus or bacteria). Thus, a vaccine is a specific remedy to the invaders by which a living body is defended against a mark molecule because both innate and acquired immunities are activated. During the process, T-cells and B-cells are activated through dendritic cells, and antibody production or cytotoxicity is induced by targeting a foreign pathogen (bacteria and virus). When our immunity function receives an antigen stimulus in the first time, high antibody value is detected by whole serum about 1 week later, but this is transient and production of an antibody is suspended gradually (the first reply). When the same antigen stimulus is received again, an IgG antibody more expensive than the first time is produced quickly, the continuation, it becomes longer (secondary response). Using the special quality of such immunity, we make them reply secondarily by additional inoculation by a vaccine, and often expect to make them maintain high antibody value and a strong immunity memory (the booster effect). It is usually classified by a live vaccine and an inactivated vaccine under a vaccine big. To use living virus and bacteria for a live vaccine will show the same reaction as the explanation a pathogen invaded. The high immunoreactive effect is obtained; however, the danger infected with the disease is also high though it is a natural thing. An inactivated vaccine loses and uses a causal factor of the disease and so you assume that safety is high. Thus, we prefer to use an inactivated vaccine for the therapeutic vaccines for chronic diseases. In the treatment of

vaccine, adjuvants are often used to raise an immune response in an inactivated vaccine; however, it sometimes causes allergic reaction. Adjuvants, such as an aluminum hydroxide, are usually added for the purpose of strengthening the kind of antigens, the amount, and the case when enough immunoresponse is not obtained by prescription method. An immune response by a vaccine is classified as humoral immunity (something by which B-cells differentiate into plasmacytes and produces a peculiar antibody in an antigen) and cellular immunity (something by which peculiar making susceptible T-cells is led and carries cytotoxicity in an antigen). A live vaccine strongly induces cellular immunity to a pathogen, which can be mainly defended by cytotoxic effect; an inactivated vaccine is effective in a defended pathogen, which is neutralized by an antibody because hormonal immunity is activated by an inactivated vaccine with adjuvants (Akira 2011).

1.3 Active Immunotherapy for an Endogenous Protein

A general concept to therapeutic vaccines is assumed for a pathogen of foreignness or a cancer cell. When considering the endogenous protein which exists in the living body like a high blood pressure vaccine as a mark molecule, it is necessary to assume the different situation in terms of both efficiency and safety aspects. Immunotolerance consists with central tolerance (negative selection) and peripheral tolerance (anergy) in our immunity system because it immunologically operates to nonreact to an endogenous protein for our living body. Central T-cell tolerance blocks the egress of self-reactive T-cells from the thymus, whereas peripheral tolerance is based on inactivation of T-cells by induction of "anergy." When the immune response is induced, T-cells usually recognizes amino acid sequence of the MHC class I or class II (read as antigen sequence) shown to the film surface of the antigen-presenting cell (dendritic cells) with the CD28/B7 interaction in the surface of dendritic cells and T-cells, which leads to T-cell monoclonal expansion (Fig. 1.1). It indicates that there are two important signals between the dendritic cells and T-cells. If only one signal (the display of antigen in MHC class I or II) was induced without co-stimulation of the CD28/B7 interaction, T-cell proliferation will be rapidly terminated, which is the mechanism of peripheral tolerance called anergy. Therefore, the key step to activate T-cells is upregulation of CD28 in dendritic cells, which is induced by the activation of innate immunity. The invaders (i.e., virus or bacteria) usually activate innate immunity which may nonspecifically react to the invaders, leading to the upregulation of CD28 in dendritic cells. However, the treatment of vaccine using endogenous protein does not activate the innate immunity and T-cell proliferation due to peripheral tolerance. Interestingly, self-reactive B-cells for an endogenous protein are still active during the immunotolerance, and the efficient antibody production by B-cells (plasmablasts) requires helper T-cell activation (Wardemann et al. 2003). Thus, the major mechanism for immunotolerance is driven by T-cells including central and peripheral tolerance. Of importance, the co-treatment with adjuvants for endogenous protein can break down the peripheral

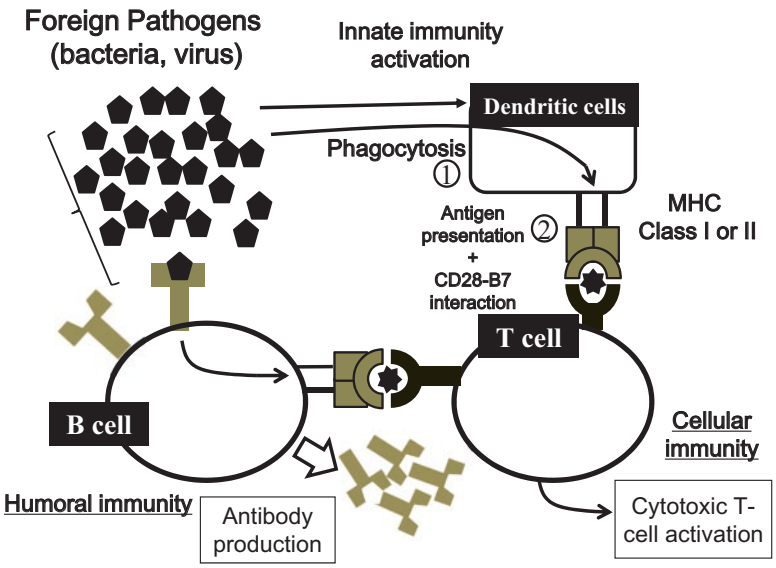

Fig. 1.1 Typical immune response against foreign pathogens
Foreign pathogens (i.e., bacteria, virus) strongly induce the activation of innate immunity, which leads to the CD26-B7 interaction between dendritic cells and T-cells (step 1). The dendritic cells phagocytose the pathogen and present a T-cell epitope to T-cells through the major histocompatibility complex (MHC). Co-activation of innate immunity and antigen presentation may induce T-cell activation, resulting in both cytotoxic T-cell and antibody production (step 2)

immune tolerance because adjuvants induce the activation of innate immunity. When the innate immunity system is activated by adjuvants, T-cells will be switched on by manifested increase as a result of the CD28/B7 interaction. After that, the stimulus is transmitted to B-cells from activated helper T-cells, and an antibody production for an endogenous protein is produced (Bachmann and Kopf 1999) In this therapeutic vaccine for an endogenous protein, overactivation of cellular and hormonal immunities might be a risk to gain a possibility of destroying its own cell. In addition, if the endogenous protein itself amplified the immune response following by vaccination, the immunoreaction will not be systemically under control. In a clinical trial for Alzheimer's disease, the use of a vaccine targeting beta amyloid as a self-antigen was halted because the participants developed aseptic meningoencephalitis, due to autoimmune response (Ferrer et al. 2004). Thus, immunological reactions should be more thoroughly considered when designing therapeutic vaccines targeting self-antigens. After vaccine administration, phagocytic, antigen-presenting cells (APCs) present epitopes to T-cells through the major histocompatibility complex (MHC). In our system, we preferred to select short antigen peptides that do not include a T-cell epitope. As MHC class I and II epitopes usually consist of 8–10 and 10–20 amino acids, respectively, short peptides with fewer than 8 amino acids are preferred as antigens, for safety issues. Thus, it is necessary to devise the vaccine system to avoid the wrong reply.

1.4 Design of Angiotensin II Vaccine for High Blood Pressure

Angiotensin II is an endogenous hormone consisting eight amino acids, and the therapeutic vaccine has been designed to induce the production of Ang II antagonizing antibodies without causing a cytotoxic immune response. As we mentioned above, the reaction against Ang II is tightly controlled via the repression of self-reactive T-cells (immune tolerance). To fully activate B-cells, CD4+ T-cells must first differentiate into plasma and memory cells. Because of T-cell immune tolerance, self-reactive B-cells, albeit responsive to antigens, cannot function without the help of CD4+ T-cells, targeting the self-antigen. As angiotensin II do not include the T-cell epitope, T-cells cannot be activated by angiotensin II itself, and B-cell-induced antibody production does not occur. To overcome this problem, carrier protein (i.e., keyhole limpet hemocyanin (KLH)) is utilized which includes foreign T-cell epitopes. Based on these devises, the mice or rats were immunized with the Ang II-KLH conjugate, with the addition of adjuvants, to circumvent T-cell tolerance (Fig. 1.2). During the immunization phase, APCs internalize Ang II-KLH and present a KLH-derived T-cell epitope to T-cells, which become activated (i.e., differentiate into effector T-cells). Importantly, Ang II itself is not presented to T-cells

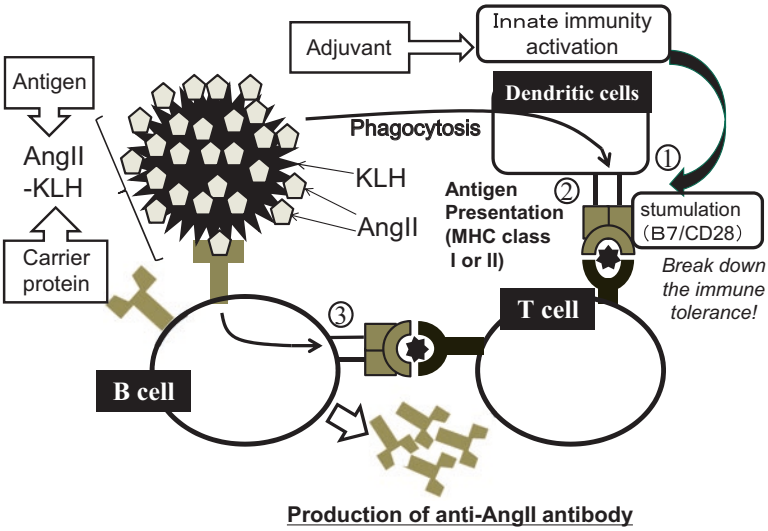

Fig. 1.2 Conceptual schematic of therapeutic vaccines for self-angiotensin II
The co-treatment of adjuvants effectively induces the CD28-B7 interaction through the activation of innate immunity (step 1). The dendritic cells (antigen-presenting cells: APCs) phagocytose the angiotensin II-carrier (KLH) conjugate and present a T-cell epitope of KLH to T-cells through the major histocompatibility complex (MHC) (step 2). Thus, T-cells recognize the epitope through T-cell receptors and become activated. B-cells specifically recognizing the target antigen differentiate into plasmacytes and proliferate with the help of activated T-cells. B-cells then produce anti-Ang II antibodies (step 3). Because Ang II alone does not include a T-cell epitope, cytotoxic T-cells are not activated for Ang II and do not attack the angiotensinogen-producing cells

through the MHC. When the MHC is recognized, T-cells do not receive co-stimulation as a result of the CD28/B7 interaction, leading to T-cell anergy. However, the adjuvant effectively induces the CD28/B7 interaction via the activation of innate immunity. Thus, the combination of Ang II-KLH and adjuvants successfully induces proliferation and differentiation of T-cells against Ang II-KLH. Ang II-specific B-cells internalize the Ang II-KLH complex and present the T-cell epitope of Ang II-KLH to T-cells. B-cells then differentiate into plasmacytes and produce antibodies with the help of effector T-cells (Nakagami and Morishita 2018). Since Ang II does not include a T-cell epitope, cytotoxic T-cells are not activated by Ang II and do not attack the angiotensinogen-producing cells. The described approach strongly induced anti-Ang II antibodies, without cytotoxic T-cell activation. T-cell proliferation and Enzyme-Linked ImmunoSpot (ELISPOT) assays were conducted to confirm the results and identify the involved T-cell isotopes (Nakagami et al. 2013). The results showed that Ang II-KLH and KLH alone induced T-cell activation, while Ang II alone did not, suggesting that only KLH contains a T-cell epitope. To further assess our results, the presence of self-antibodies was evaluated after continuous infusion of Ang II. Interestingly, no increase in the titer of anti-Ang II antibodies was detected in immunized mice. Therefore, this vaccine system did not induce an autoimmune reaction, due to the "nonself" recognition of Ang II-KLH and KLH foreign T-cell epitopes.

1.5 Conclusion

As shown in Fig. 1.3, the therapeutic vaccine for an endogenous protein has three key factors (antigen setting, carrier protein, and adjuvants) to induce the specific antibody production. In case of therapeutic vaccine for chronic diseases, it is desirable that an antigen excludes MHC class I and II arrangement (T-cell epitopes) from antigen sequence when antigen setting itself does not activate T-cells. This antigen setting is different from the vaccine for infectious disease or cancer. Their antigen usually includes T-cell epitopes to directly activate T-cells, which leads to activating cytotoxic T-cells (cellular immunity). In case of therapeutic vaccine, carrier protein, which includes the T-cell epitope sequence, is really required instead because activation of helper T-cells is needed for stable antibody production from T-cells. For this purpose, KLH and VLP (particle like a virus) are generally used as a carrier in these vaccines (Paul et al. 1974, Jennings and Bachmann 2009).

In terms of adjuvant selection, our therapeutic vaccine is substantially different from the previous ones. For "traditional" vaccines, requiring activation of cytotoxic T-cells, adjuvants that activate the Th1 pathway and involve the production of IFN-γ are preferred, such as CpG. As the therapeutic vaccine aims at inducing antibody production without cytotoxic T-cell activation, adjuvants promoting the Th2 pathway are more appropriate (i.e., Alum). In addition, our vaccine may induce IgG2, which has no effector functions. The correct combination of carriers and adjuvants is critical for managing safety issues during the development of therapeutic vaccines (Koyama et al. 2009; Kuroda et al. 2013).

Target diseases	Cancer, Infectious Diseases	Chronic Diseases (Hypertension, Alzheimer's diseases)
Goal	Cytotoxic T-cell activation Antibody production (effector function)	Antibody Production (neutralizing function)
Vaccine type	Live vaccine DNA or recombinant protein vaccine	Inactivate vaccine DNA or peptide vaccine
Antigen	Include MHC class I or II (T-cell epitope)	Exclude MHC class I or II (B-cell epitope)
Carrier Protein	Not necessary	Important (KLH, VLP)
Adjuvants	Th1 direction (CpG oligonucleotide)	Th2 direction (Alum)

Fig. 1.3 Comparison of vaccines for cancer, infectious diseases, and chronic diseases
The goal of vaccines against cancer or infectious disease is the activation of cytotoxic T-cells. Live vaccines are sometimes utilized for prevention of infectious diseases. Their antigens should present a T-cell epitope through the MHC, and a carrier is not required for this system. Therefore, these vaccines use adjuvants that activate the Th1 pathway (i.e., CpG), which involves the production of IFN-γ. For chronic diseases, the goal is the induction of antibodies without cytotoxic T-cell activation. Nonactivated vaccines are usually utilized for this purpose. In this case, antigens should exclude a T-cell epitope, and a carrier is utilized to provide for the foreign T-cell epitope instead of antigen. Adjuvants that activate the Th2 pathway (i.e., Alum) are preferable for this type of vaccine

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Chapter 2
Therapeutic Vaccines Targeting Alzheimer's Disease

Shuko Takeda

Abstract The rapid increase in patients with dementia has become a global social problem. The most common cause of dementia is Alzheimer's disease, accounting for more than half of all reported cases. At present, disease-modifying therapy for Alzheimer's disease has not been established, and thus current treatments consist of symptomatic therapy drugs that only temporarily improve clinical symptoms. Characteristic pathological changes, i.e., senile plaques (extracellular aggregates of β-amyloid) and neurofibrillary tangles (intracellular aggregates of tau), appear in brains of patients with Alzheimer's disease. Although the pathophysiological mechanism of the disease has not been sufficiently elucidated, it is suggested that these pathological proteins play important roles in neuronal dysfunction. In recent years, attempts to develop immunotherapies targeting these pathological proteins have become active all over the world. The development of an immunotherapy targeting Aβ is ongoing; however, most clinical trials have failed, and thus its effectiveness has not yet been proven. Recently, the development of an immunotherapy targeting tau has also advanced, yielding promising results in animal experiments. In this chapter, we will review the latest findings and future prospects on the development of immunotherapies targeting pathological proteins related to Alzheimer's disease.

Keywords Dementia · Alzheimer's disease · Aβ · Tau · Immunotherapy · Vaccine

S. Takeda (✉)
Department of Clinical Gene Therapy, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan
e-mail: takeda@cgt.med.osaka-u.ac.jp

H. Nakagami (ed.), *Therapeutic Vaccines as Novel Immunotherapy*,
https://doi.org/10.1007/978-981-32-9628-2_2

2.1 Introduction

The rapid increase in patients with dementia has become a global social problem. Alzheimer's Disease International (ADI) issued a report stating that the number of dementia patients in the world may reach 132 million by 2050, three times as many as at present (about 46.8 million) (Fiest et al. 2016; Livingston et al. 2017). The number of new patients is estimated to be roughly 9.9 million every year, which corresponds to an increase of 1 case every 3.2 s. This estimate is 30% higher than that in 2010, and the number is expected to further increase rapidly along with the aging population. Currently, the number of elderly people over the age of 60 worldwide is estimated to be 900 million or more. This aged population will likely increase by 65% in rich countries, 185% in middle and low income countries, and 239% in poor countries over the next 35 years. Regionally, the increase in dementia among aging individuals is most prominent in Asia. The medical and economic burdens associated with these statistics pose serious problems in poor countries (Livingston et al. 2017).

Alzheimer's disease (AD) is the most common cause of dementia, accounting for more than half of all cases (Karran et al. 2011). AD is a progressive disease, initially manifesting as memory impairment, then gradually incorporating symptoms such as aphasia and visuospatial deficit, before eventually leading to a bedridden state and early death. As the stages progress, the burdens for caregivers and family also increase.

Two distinctive neuropathological changes, i.e., senile plaque and neurofibrillary tangle, appear in the AD brain (Serrano-Pozo et al. 2011). Senile plaques form through the deposition of aggregated β-amyloid (Aβ) in the brain parenchyma, while neurofibrillary tangles appear through the aggregation of tau in neuronal cells (Fig. 2.1). Senile plaques and neurofibrillary tangles have been shown to accumulate in the brain several decades prior to the onset of clinical symptoms (Bateman et al. 2012). This suggests that the accumulation of Aβ and tau is the fundamental cause of AD (Selkoe 2000). Therefore, there are many ongoing efforts to develop therapeutic methods capable of targeting these two pathological proteins.

2.2 Immunotherapy Targeting Aβ

Pathological proteins Aβ and tau accumulate in the AD brain; Aβ aggregation and accumulation are known to precede tau tangle formation. This suggests that the accumulation of Aβ induces that of tau, thereby causing neurological dysfunction. This scenario is called "amyloid cascade hypothesis."(Selkoe and Hardy 2016) This postulates that the most fundamental cause of AD is an accumulation of Aβ. On this basis, therapeutic methods to reduce Aβ in the brain are being actively explored as promising disease-modifying therapy for AD.

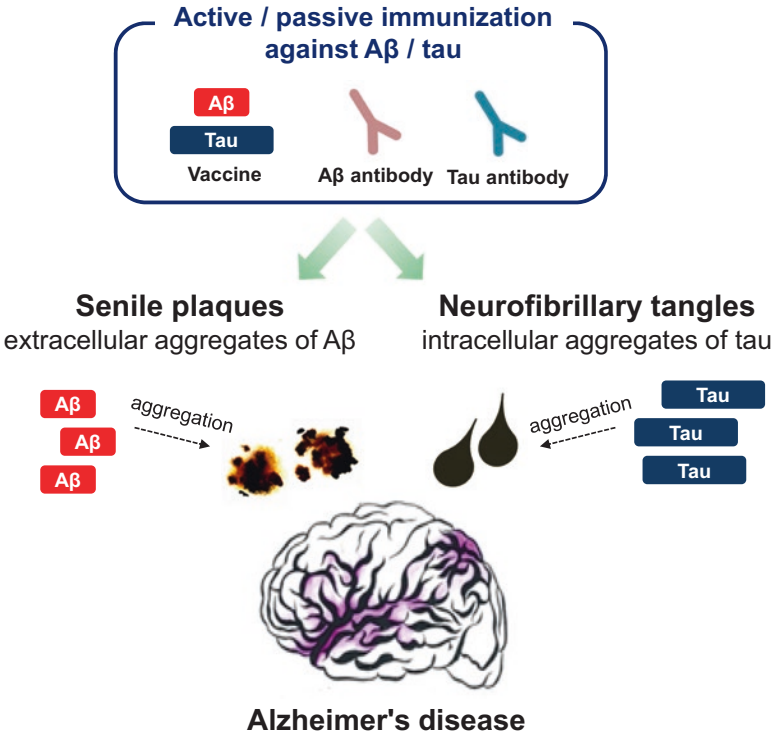

Fig. 2.1 Immunotherapy targeting AD-related neuropathologies
AD is characterized by two neuropathological hallmarks, senile plaques (extracellular aggregates of) and neurofibrillary tangles (intracellular aggregates of tau). Aβ and tau are neurotoxic and play important roles in the AD pathogenesis. The development of active and passive immunotherapies targeting these pathologic proteins is ongoing. Aβ, β-amyloid

It was previously believed that antibodies could not pass through the blood-brain barrier (BBB); therefore, immunotherapy was not considered promising as a method to remove pathological proteins in the brain (Bien-Ly et al. 2015; Pedersen and Sigurdsson 2015; Congdon and Sigurdsson 2018). However, Schenk et al. demonstrated the potential effectiveness of immunotherapy for AD in 1999 via experiments using an AD mouse model for the first time (Schenk et al. 1999). They reported that amyloid plaques in the brains of AD mouse model disappeared after inducing immune responses by administering Aβ42 peptide. This result was confirmed by other groups of researchers, who showed that not only had amyloid plaques in the brains of AD mice disappeared (Spencer and Masliah 2014), but also that their cognitive dysfunction improved (Bard et al. 2000; Das et al. 2001).

Following these promising results in animal experiments, clinical trials of Aβ vaccine therapy for human AD patients started in 2001 (Lemere and Masliah 2010). In clinical trial AN1792, full-length Aβ42 was used as an active immunization agent; however, severe encephalitis developed in about 6% of the patients treated,

some of whom died, leading to the discontinuation of the trial. Subsequent analysis confirmed that in some cases the senile plaques in the brain disappeared among patients who received the vaccine treatment, indicating a possibility that vaccine therapy could be a viable approach (Nicoll et al. 2003). However, it was found that cognitive dysfunction was advanced even in patients whose senile plaques had disappeared (Holmes et al. 2008), challenging the significance of Aβ as a target for therapies.

It is thought that these trials might have failed because the accumulation of Aβ had already reached maximum levels when clinical symptoms of dementia appeared, and thus the treatments started too late to be effective (Selkoe 2013). This has shifted the strategy to starting the therapy targeting Aβ at earlier stages. Advances in diagnostic methods using positron emission tomography (PET) imaging and cerebrospinal fluid biomarkers have enabled researchers to recognize the presence of Aβ pathology before the development of clinical symptoms (Bateman et al. 2012). The efficacy of the treatment at earlier stages when Aβ accumulation has not progressed so much is currently being examined in clinical trials.

It is also known that neurotoxicity varies with different species of Aβ (Lee et al. 2017), suggesting that the immunotherapies to date could not remove highly toxic Aβ species. Aβ peptides are 40–42 amino acids long, of which Aβ42 is thought to be highly aggregating and neurotoxic (Hashimoto et al. 2012; Arbel-Ornath et al. 2017). Aβ gradually aggregates from monomers into dimers, oligomers, and fibrils, eventually forming senile plaques. Recent studies have shown that oligomers are the most neurotoxic species; therefore, the development of immunotherapies targeting Aβ oligomers is in progress (Spencer and Masliah 2014). Takamura and colleagues have shown that oligomer Aβ is a highly neurotoxic species by using an AD mouse model and autopsied human brain tissues (Takamura et al. 2011). Tomiyama and colleagues have shown that the patients with amyloid precursor protein (APP) gene mutation, which increases oligomer Aβ, develop dementia in spite of the absence of senile plaques in their brains (Tomiyama et al. 2008). Recent clinical trials targeting Aβ have shown that antibodies such as creneuzumab have affinities for oligomer Aβ (Adolfsson et al. 2012). Although the specificity of this antibody for oligomer Aβ has not been adequately verified, the results of its clinical trials are awaited (Spencer and Masliah 2014).

2.3 Immunotherapy Targeting Tau

Tau is a microtubule-associated protein that regulates the stabilization of axonal microtubules (Grundke-Iqbal et al. 1986; Mandelkow et al. 1995, 1999; Iqbal et al. 2016). The tau protein is expressed by a gene located on the long arm of chromosome 17. The NFTs of the AD brain are composed of tau and appear ultrastructurally as paired helical filaments and straight filaments (Lewis and Dickson 2016).

Tau in NFTs is aberrantly misfolded and abnormally hyper-phosphorylated (Fitzpatrick et al. 2017). The tau protein can undergo post-translational modification, which are implicated in the pathogenesis of AD (Spires-Jones et al. 2011; Simic et al. 2016). Phosphorylation alters the conformation of tau and regulates its biological activity (Morris et al. 2015). Phosphorylation is also believed to trigger aggregation leading to NFT formation. The number of NFTs shows a better correlation with neuronal loss in the AD brain than does the number of senile plaques (Gomez-Isla et al. 1997; Ossenkoppele et al. 2016). This suggests that tau directly contributes to the AD-associated neurodegeneration (Gomez-Isla et al. 1997). Tau-targeting treatments, such as aggregation inhibitors, have been under investigation, and some have shown promise in animal models (Iqbal et al. 2018). A number of clinical trials targeting tau are now ongoing (Panza et al. 2016).

Recently, the development of immunotherapies targeting tau has become active, since several attempts to develop immunotherapies targeting Aβ failed in sequence (Pedersen and Sigurdsson 2015). Experiments using a mouse model showed that Tau-transgenic mice administered with anti-tau antibodies exhibited improved tau pathology in the brain as well as improved cognitive functions (Yanamandra et al. 2013). Although it is unclear which tau species is highly neurotoxic, phospho-tau specific and anti-oligomer-tau antibodies have been shown to be effective (Takeda et al. 2015; Nobuhara et al. 2017).

It is generally accepted that antibodies cannot enter cells. Given that tau is an intracellular protein and neurofibrillary tangles are also intracellular aggregates, it is unknown why anti-tau antibodies can act on intracellular targets. Meanwhile, the importance of extracellular tau has been demonstrated in recent years. Tau can be physiologically released from cells and taken up by other nerve cells to form new tau pathologies. This phenomenon, referred to as "tau propagation," has drawn attention as a new therapeutic target for AD (Frost and Diamond 2010). Takeda and colleagues have reported that they identified a specific species of tau that is involved in tau propagation, which could be a potential target for AD treatment (Takeda et al. 2015, 2016).

Tau is a protein of 441 amino acids long with a molecular weight of 55 kDa. It remains to be elucidated which epitope(s) is the most effective target(s) of immunotherapy. In the ongoing development of immunotherapies targeting tau, different pharmaceutical companies use different epitopes in N-terminal, mid-domain, or C-terminal regions as a target (Pedersen and Sigurdsson 2015). Recent analysis using an in vitro assay system has compared seven different antibodies and shown that those targeting the N-terminal region of tau, as well as phospho-tau, are effective in blocking tau propagation (Nobuhara et al. 2017).

Ongoing clinical trials on tau immunotherapy are summarized in Table 2.1. There are at least two active- and six passive-immunotherapy human trials ongoing. Most of them are targeting AD, although two clinical trials are evaluating safety and efficacy in patients with progressive supranuclear palsy, another tau-related neurodegenerative disorder, as well.

Table 2.1 Ongoing clinical trials on tau immunotherapy

Active/passive	Name	Company	Epitope	Subjects	ClinicalTrials.gov Identifier	Status
Active immunotherapy	AADvac1	AXON neuroscience	Tau294–305	AD	NCT02579252	Phase II
Active immunotherapy	ACI-35	AC immune SA, Janssen	pS396 and pS404 (liposome-based vaccine)	AD	Not available	Phase Ib
Passive immunotherapy	RO7105705	AC Immune SA, Genentech, Hoffmann-La Roche	N-terminus of all six isoforms of human tau	AD	NCT02820896	Phase II
Passive immunotherapy	LY3303560	Eli Lilly & Co.	Conformational epitope primary in N-terminal region	AD	NCT03518073	Phase II
Passive immunotherapy	BIIB092	Biogen, Bristol-Myers Squibb	Extracellular, N-terminally fragmented forms of tau	AD, PSP	NCT03352557	Phase II
Passive immunotherapy	ABBV-8E12	AbbVie, C2N Diagnostics	Tau25–30	AD, PSP	NCT02880956	Phase II
Passive immunotherapy	UCB0107	UCB S.A.	Tau235–246	HS	NCT03464227	Phase I
Passive immunotherapy	JNJ-63733657	Janssen	Mid-region of tau	AD	NCT03375697	Phase I

AD Alzheimer's disease, *PSP* Progressive supranuclear palsy, *HS* healthy subjects

2.4 Active Tau Vaccine

Although the first active vaccine therapy using Aβ (AN1792) was discontinued due to severe encephalitis, active tau vaccine shows promise in animal models and human studies (Pedersen and Sigurdsson 2015; Congdon and Sigurdsson 2018). There are currently two clinical trials with active immunization approach underway (Sigurdsson 2018).

AXON neuroscience (Bratislava, Slovak Republic) initiated a phase I clinical trial evaluating its active tau vaccine (AADvac1) targeting AD. They identified important regulatory domains on tau which plays a role in pathological tau oligomerization (Kontsekova et al. 2014). AADvac1 comprises a tau fragment (294KDNIKHVPGGGS305) coupled to the keyhole limpet hemocyanin (KLH) carrier via N-terminal cysteine residue and administered with an Alhydrogel alum adjuvant. Active vaccination using AADvac1 reduced toxic tau oligomers and neurofibrillary pathology in a rat model of AD. Notably, the tau vaccine reduced pathological hyperphosphorylation of tau by 95% with improvement of clinical phenotypes. Given the good efficacy and safety profile of AADvac1 in rodent models, they started a first-in-man phase I study at multiple centers in Austria (Novak et al. 2017). Patients with mild-to-moderate AD received three doses of AADvac1 or placebo. The vaccine showed a favorable safety profile with excellent immunogenicity, encouraging a further development.

AC Immune (Lausanne, Switzerland) developed the liposome-base tau vaccine (ACI-35) (Nicolau et al. 2002). They adapted a liposome-based vaccine technology (Nicolau et al. 2002), incorporating a short form of phosphorylated tau into liposomes. The vaccine contained a 16-mer synthetic tau fragments (a.a. 393–408), with phosphorylated residues S396 and S404, which mimics pathological tau epitopes. This was anchored into a lipid bilayer and mixed with the adjuvant monophosphoryl lipid A (MPLA). The liposome-based active tau vaccine induced a rapid increase in IgG antibody levels specifically against phosphorylated tau species when injected into a mouse model of AD (Theunis et al. 2013). The vaccine improved tau pathologies and neurological deficits of the AD mice with no apparent signs of toxic neuroinflammation or other side effects. AC Immune initiated a phase I study in December 2013 evaluating ACU-35 targeting AD. This trial was registered at multiple institutions in Finland and UK. Patients with mild-to-moderate AD received low, medium, and high doses of ACI-35 or placebo, with a subsequent booster shot. The aim of this study is to test the safety and immunogenicity, but cerebrospinal biomarkers and cognitive scores are also evaluated during the study period. This was the first human trial targeting phosphorylated tau epitopes.

2.5 How Do Antibodies Enter the Brain and How Do They Remove Pathological Proteins?

The effectiveness of immunotherapies for pathological proteins in the brain such as Aβ and tau has been demonstrated in mice and humans; however, the mechanisms underlying these immunotherapies are not fully understood. How antibodies that were previously thought incapable of crossing the BBB can indeed enter the brain and bind to pathologic proteins, and how said proteins are subsequently removed, remains poorly explored.

Although antibodies are believed not to pass the BBB, some sites in the brain, such as circumventricular organs, physiologically lack BBB (Wilhelm et al. 2016); therefore, antibodies may enter the brain through these sites. The BBB often breaks due to cerebrovascular arteriosclerosis or the cerebrovascular dysfunction effects due to Aβ in elderly people who develop dementia (Takeda et al. 2014), which likely allows antibodies in the blood to enter the brain. It has been reported that the binding of antibodies to Aβ promotes its degradation through phagocytosis by microglia. One hypothesis is that anti-Aβ antibodies in the blood direct Aβ proteins in the brain into the blood (sink theory); however, its occurrence in the human brain has not been confirmed (Morgan 2005; Deane et al. 2009).

These points need to be clarified for future successful immunotherapy targeting Aβ or tau. For example, elucidating the mechanism by which antibodies in the blood enter the brain, followed by the development of a method to promote it, could enhance the effects of conventional immunotherapies.

2.6 Conclusion

It was believed that antibodies could not enter the brain from the blood, since the brain is separated from peripheral blood vessels by the BBB. However, recent studies using animal experiments have shown that immunotherapy can decrease the pathological proteins (Aβ and tau) that play roles in the AD pathogenesis, indicating that antibodies can indeed act on these pathological proteins in the brain in some way. Because antibodies can specifically capture pathological proteins that contribute to diseases, they are likely to be effective for diseases whose therapeutic targets are defined, as is the case with AD. AD develops with prolonged courses and thus requires a large number of doses, which may increase medical expenses. Vaccine therapy is likely to become a promising counter for dementias like AD as a less expensive immunotherapy.

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Chapter 3
A Vaccine for Ischemic Stroke

Munehisa Shimamura, Tomohiro Kawano, Kouji Wakayama, and Hironori Nakagami

Abstract Poor adherence to secondary prevention in poststroke patients is one of the major causes for recurrence of ischemic stroke. Vaccination is a promising strategy for overcoming this limitation because of its long-term effects; however, it may have an increased risk of long-term side effects as well. Nevertheless, our recent studies reported that a vaccine against S100A9 and Ang II was effective without causing any major side effects. In this chapter, we present the history of vaccination for ischemic stroke and the possible use of antithrombotic and antihypertensive vaccines as secondary prevention measures in poststroke patients.

Keywords Vaccine · Thrombosis · Ischemic stroke · Hypertension

Although secondary prevention in ischemic stroke is important to avoid recurrence of attack, several studies have reported that patients discontinue the medication. For example, a study in Sweden reported that 36.3% or 55% of patients discontinued antiplatelet drugs or warfarin, and 25.8% of patients did not continue the use of antihypertensive drugs till 2 years (Glader et al. 2010). Discontinuation of the antiplatelet therapy within 1 year was also reported in 22.5% of the aspirin-treated, 35.8% of clopidogrel-treated, and 31.8% of warfarin-treated patients in the United States (Bushnell et al. 2011). In Japan, approximately 40% of patients diagnosed

M. Shimamura (✉) · T. Kawano
Department of Health Development and Medicine, Osaka University, Graduate School of Medicine, Suita, Osaka, Japan

Department of Neurology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan
e-mail: shimamuu@cgt.med.osaka-u.ac.jp

K. Wakayama
Department of Advanced Clinical Science and Therapeutics, University of Tokyo, Tokyo, Japan

H. Nakagami
Department of Health Development and Medicine, Osaka University, Graduate School of Medicine, Suita, Osaka, Japan

H. Nakagami (ed.), *Therapeutic Vaccines as Novel Immunotherapy*,
https://doi.org/10.1007/978-981-32-9628-2_3

with recurrent ischemic stroke had discontinued their antithrombotic medication (Ito et al. 2011). The reason for discontinuing the medication included several factors such as gender, medical history, education level, number of medications upon discharge, poor self-perceived general health, or depression after ischemic stroke (Glader et al. 2010). As vaccines are effective for long periods and include several injections, they could be a promising approach in order to improve poor adherence to secondary medicine; however, the presumable long-lasting side effects are a major concern. A common side effect is harmful immune response; moreover, disease-specific side effects include a long-lasting risk of hypotension in case of antihypertensive vaccines and bleeding in case of antithrombotic vaccines. Another problem is that it takes several weeks to get sufficient antibody titer. This is especially problematic for secondary prevention, and a period involving combination therapy with a vaccine and an existing medicine might, therefore, be required. Moreover, the interference of the blood–brain barrier should be considered when the antibody produced is effective only in the parenchyma because IgG antibodies could cross the intact BBB at very low rates (Banks et al. 2002).

In this chapter, the advantages and limitations of vaccination in ischemic stroke are discussed.

3.1 History of Vaccine in Ischemic Stroke

Vaccines as well as antibodies against specific proteins are used to induce immune tolerance. Early vaccine experimentation in stroke aimed to prevent ischemic injury by inducing immune tolerance. Immune tolerance is defined as unresponsiveness to an antigen that is induced via previous exposure to that antigen. When specific lymphocytes encounter antigens, the lymphocytes may be activated, leading to immune responses, or the cells may be inactivated or eliminated, leading to tolerance. Various forms of the same antigen may induce an immune response or tolerance (KA and HL 2003). Although the mechanism inducing immunity or immune tolerance via injected molecules is still controversial, it is assumed to be dependent on factors such as signals that T cells receive from the antigen-presenting cells (APCs) or environmental factors during the activation process. Before the vaccine experiment in stroke, a vaccine targeting immune tolerance was extensively studied in multiple sclerosis (MS), where autoimmunity played a pivotal role in the susceptibility and development of the disease. Although the autoantigens in MS remain controversial, the autoreactive T cells against myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) were suggested to be immunological antigens via animal model studies (experimental autoimmune encephalomyelitis [EAE]) (Wekerle et al. 1994). Vaccines inducing immune tolerance for MOG or MBP were known to be effective in EAE models by the intradermal or intramuscular injection of plasmid DNA coding MBP or MOG (Shimamura et al. 2011) or the peptides of these mol-

ecules (Willekens and Cools 2018). Thereafter, the oral mucosa immune tolerance was checked in rodent models with ischemic stroke (Becker et al. 1997; Gee et al. 2008, 2009). Immunologically, an inflammatory response is evoked in the brain and its periphery during the early stage of stroke, wherein the immune system later exerts an immunosuppressive effect to limit the development of an autoimmune response to the brain antigens exposed to the immune system due to brain injury (Dirnagl et al. 2007; Gee et al. 2009; Iadecola and Anrather 2011). When animals are subjected to a systemic inflammation insult with an enhanced autoimmune response, they receive a worse functional outcome after middle cerebral artery occlusion (MCAO) (Becker et al. 2005). One of the causative agents of such an autoimmune response after ischemic stroke is MBP; immune tolerance for MBP was observed via oral mucosa in rodent models (Becker et al. 1997; Gee et al. 2008, 2009). Immune deviation from Th1 to Th2 T cell subsets, clonal anergy or deletion, and active suppression by TGF-β-secreting T cells were implicated as possible mechanisms in oral tolerance (Jewell et al. 1998). During the early stages of the experiment, immune tolerance for MBP prior to cerebral ischemia revealed successful reduction in the infarct volume at 24 and 96 h after ischemia (Becker et al. 1997); however, during the later stages, tolerization to MBP indicated a tendency to develop a Th1 response to MBP and the development of deteriorative effects by 3 months after MCAO. This study raised a concern about the potential for inadvertent induction of detrimental autoimmunity via mucosal administration of antigen in the chronic stage of ischemic stroke (Gee et al. 2009). Thus, the use of an immune tolerance vaccine for ischemic stroke was unsuccessful; other targets such as MOG might prove to be alternative targets as the phase II clinical trial using a DNA vaccine encoding MBP was efficient in MS (Garren et al. 2008; Juryńczyk et al. 2010).

3.2 Antithrombotic Vaccine

Antithrombotic peptide vaccines inducing antibodies for target molecules were recently reported, which differed from the immune tolerance vaccine (Zhong et al. 2017; Kawano et al. 2018). The antithrombotic agents are generally classified as anticoagulants, such as warfarin or direct oral anticoagulants (DOAC) and antiplatelet agents, such as aspirin, extended-release dipyridamole, and clopidogrel. For the secondary prevention of ischemic stroke, the former is used for cardioembolic stroke and the latter is used for noncardioembolic stroke. Although various anticoagulant and antiplatelet agents target different molecules in the thrombosis pathway, a majority of agents cause increased risk of bleeding. For example, the direct thrombin inhibitor, dabigatran, and the direct factor Xa inhibitors, such as apixaban, rivaroxaban, and edoxaban, are used for secondary prevention in cardioembolic stroke; however, they increase the risk of bleeding (Koenig-Oberhuber and Filipovic 2016). To avoid such hemorrhagic risks, a peptide vaccine targeting factor XI (FXI) (Zhong

et al. 2017) was recently reported in the rodent models. Because the gene knockout mice of FXI and epidemiologic observations revealed that FXI is crucial in hemostasis (Bane and Gailani 2014; Chen et al. 2014; Gailani et al. 2015) and an FXI antisense oligonucleotide indicated prevention in postoperative venous thrombosis without increasing the risk of bleeding in clinical II trials (Büller et al. 2015), FXI will be an ideal target of antithrombotic vaccine. The study on this vaccine reported that the vaccine induced a significant antibody response against FXI and reduced the plasma FXI activity; moreover, the antithrombotic effects were seen in several venous thrombosis models (Zhong et al. 2017). Nevertheless, the study lacked the evaluation of the bleeding risk, immunologic responses, and the long-term efficacy after FXI vaccine. If these are clarified in the future study, FXI will be preferred for the antithrombotic vaccine.

In antiplatelet agents, aspirin and P2Y12 receptor antagonist (clopidogrel) are used in secondary prevention of ischemic stroke. In addition to these, prasugrel, tocagrelor, and other glycoprotein IIb/IIIa inhibitors (abxicimab, tirofiban, and eptifibatide) are administered for the heart diseases. Nevertheless, all these increase the bleeding risk (Koenig-Oberhuber and Filipovic 2016). Differing from these agents, protease-activated receptor (PAR)-1 inhibitor is an antiplatelet medicine, which was reported to prevent thrombotic formation without affecting the coagulation and bleeding time in animal models (Cai et al. 2015) and phase 2 clinical trials (Goto et al. 2010). However, patients who received PAR-1 inhibitor in addition to standard of care antiplatelet therapy were reported to have an increased incidence of bleeding events compared with the placebo in phase 3 trials (Tricoci et al. 2012; Morrow et al. 2012). As aforementioned, the combination of standard care and vaccine will be required until the antibody titer reaches the sufficient amount; PAR-1 might not be a suitable target for vaccine due to the bleeding risk.

Another possible target for an antiplatelet vaccine is S100A9, which is associated with thrombus formation without affecting hemostasis (Wang et al. 2014). S100A9 is one of the damage-associated molecular patterns (DAMPs) produced and secreted from damaged or dead cells in pathological conditions and forms a heterodimer with S100A8 (Schiopu and Cotoi 2013). S100A9 and S100A8/A9 heterodimer are also expressed in platelet (Fig. 3.1a). When the vasculature is injured, they are secreted from platelets and promotes thrombosis (Wang et al. 2014) (Fig. 3.1b). Although the CD36 receptor, toll-like receptors (TLRs), and the receptor for advanced glycation and end products (RAGE) are reported to the receptor for S100A9, only S100A9/CD36 was known to be a key signal in the regulation of thrombus formation (Fig. 3.1c, d) (Wang et al. 2014). In the S100A9 knockout mice, occlusion times after photochemical injury of the carotid artery were prolonged without affecting the bleeding time, and platelet thrombus formation under laminar flow was inhibited in the presence of the anti-MRP-14 monoclonal antibody 1H9. The reduced platelet activation assessed by the expression of P-selectin, and activated GPIIb/IIIa in response to agonist stimulation was also reported (Wang et al. 2014). Although the mechanism of antithrombotic effects without affecting hemostasis in S100A9$^{-/-}$ mice was not clarified, we speculated that the following points regarding this molecule are advantageous for an antithrombotic vaccine: (1)

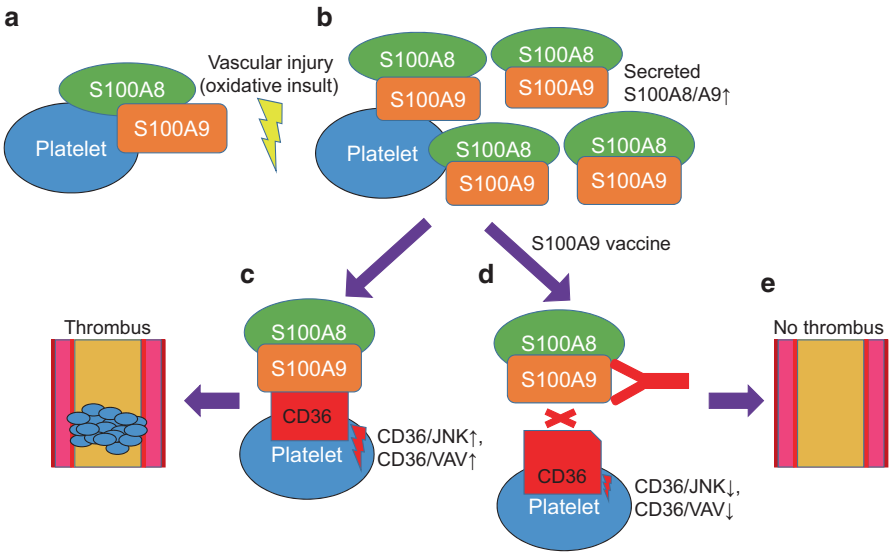

Fig. 3.1 Schematic diagram of S100A9 antithrombotic vaccine
(**a**) In normal healthy mice, S100A9 was slightly expressed in the serum. S100A9 formed heterodimer with S100A8, but S100A9 was reported to be important for thrombus formation. (**b**) S100A8/A9 was secreted from the activated platelet after vascular injury. (**c**, **d**) S100A9 subsequently promotes thrombotic formation via binding to the platelet CD36 receptor. (**e**) The autoantibodies against S100A9 in mice vaccinated with S100A9 inhibit the formation of thrombus formation by blocking the S100A9/CD36 signal

Because a previous study revealed that S100A9-null mice had a normal lifespan without abnormal organs and tissues (Hobbs et al. 2003), long-term inhibition of S100A9 by a vaccine, namely the "S100A9 vaccine," might not cause serious side effects; (2) S100A9 vaccine might also be able to reduce atherosclerotic lesions because double knockout mice that lack S100A9 and apolipoprotein E were reported to reveal attenuated atherosclerosis lesion areas and macrophage accumulation in plaques than those in apolipoprotein E knockout mice (Croce et al. 2009). Since atherosclerosis is one of major factors causing recurrent stroke, preventing the progression of atherosclerosis by S100A9 vaccine might be an additional advantage.

From the viewpoint, we designed a peptide vaccine targeting the C-terminus of S100A9 (Kawano et al. 2018). This peptide revealed antithrombotic effects via inhibition of S100A9/CD36 signal (Fig. 3.1e) and its effect was similar to that of clopidogrel (Kawano et al. 2018). S100A9 forms a heterodimer with S100A8 (S100A8/A9); however, the vaccine for S100A8 did not reveal any antithrombotic effects. The effects were observed in common carotid arteries as well as middle cerebral arteries (MCA), whose structure considerably differed from the extracranial arteries (Shimamura et al. 2013). The antibody against S100A9 was observed at least 2 months after vaccination and the effect lasted even when the antibody titer was lowered (Fig. 3.2). Furthermore, boost immunization increased the antibody titer again. Importantly, S100A9 vaccine did not affect the bleeding time, which consid-

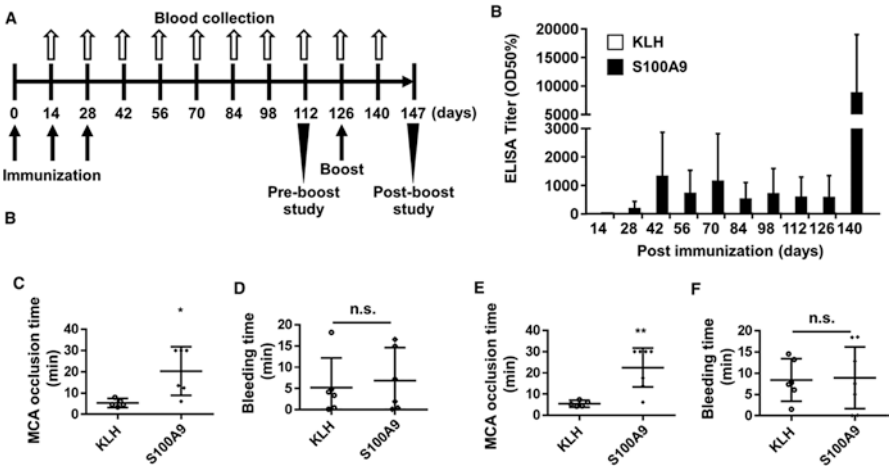

Fig. 3.2 Long-term antithrombotic effects of S100A9 vaccine in the $FeCl_3$-induced vascular injury model in middle cerebral artery (MCA) in mice (cited from (Kawano et al. 2018))
(**a**) S100A9 vaccine was injected at 0, 14, and 28 days; booster was administered at 126 days; and anti-S100A9 titer was measured every 2 weeks. (**b**) Antibody titer was increased from 42 days and was increased until 126 days. Boost immunization induced a remarkable increase in the antibody titer (KLH control, $n = 6$; S100A9, $n = 7$). (**c**, **d**) The occlusion time in MCA (**c**) and bleeding time after tail cut (**d**) before booster immunization were measured at 112 days in mice vaccinated with KLH (control, $n = 6$) and S100A9 ($n = 5$). (**e**, **f**) Occlusion time (**e**) and bleeding time (**f**) after booster immunization were measured at 147 days in mice vaccinated with KLH (control, $n = 6$) and S100A9 (50 μg, $n = 7$). $*P < 0.05$; $**P < 0.01$; n.s., not significant

erably differed from that of clopidogrel. The ELISPOT assay, IgG subclass, and histological analysis of brain, lung, and kidney did not reveal any harmful autoimmune response (Kawano et al. 2018). These results indicated that S100A9 is preferred for antithrombotic vaccine due to its long-term effect without increased risk of bleeding or harmful immune response.

Thus, S100A9 vaccine has potential for secondary prevention in ischemic stroke; however, certain issues regarding its clinical use remain unresolved. One issue is whether the antibody is produced by vaccine after ischemic stroke because immune tolerance for intrinsic molecule is observed in the chronic stage of stroke as aforementioned. To answer this question, we examined the production of antibody against S100A9 when vaccination was commenced from 7 days after MCAO in mice. In these postischemic mice, the antibody for S100A9 was successfully induced and the antithrombotic effect was observed similar to the vaccination in normal mice (Kawano et al. 2018). Thus, at least in the rodent models, vaccine for S100A9 poststroke was presumably administered. Another issue was differences in the amino acid sequence of S100A9 between mice and humans. The identity of amino acid compositions in S100A9 between mouse and human is 11.4%, and the sequence in the epitope in human correspondent to the selected epitope in our experiment (Kawano et al. 2018) is different. Because the identity is 88.6% between human and monkey (*Macaca fascicularis*), newly designed epitope vaccine for

monkey should be examined to check whether the effects observed in mice are also seen. Moreover, selection of suitable adjuvants is necessary. We used Freund's adjuvant to get maximum immune responses for the exploratory research and a clinically available adjuvant, such as alum, which could shift the immune response toward a Th2 predominance (Kool et al. 2012) to avoid possible encephalitis induced by Th1 predominance, as observed in the peptide formation in Alzheimer's disease (Pride et al. 2008). The suitable timing of vaccine administration should also be investigated. According to the guidelines in American Heart Association, administration of aspirin is recommended within 24–48 h after the onset of symptoms (Powers et al. 2018). As it requires several weeks to achieve sufficient antibody titers after vaccination, aspirin should be continued for the recommended time period. Aspirin can be immediately discontinued after achieving the necessary antibody titer; however, this is not advisable as the timing of sufficient antibody production will differ between the individuals. Therefore, few patients will experience a period of antithrombotic effects for both aspirin and S100A9 vaccine. The safe use of the combination of aspirin and S100A9 vaccine should be warranted in further studies.

In summary, exploring an ideal molecule, which promotes thrombotic formation without increasing the risk of bleeding, is essential for designing the antithrombotic vaccine in ischemic stroke. S100A9 is one such ideal molecule, and signal inhibition with this vaccine will be a novel strategy in secondary prevention after ischemic stroke although few concerns persist regarding the selection of suitable adjuvant, establishment of proof of concept in monkey, and of its use as a combination with aspirin, which should be resolved.

3.3 Antihypertension Vaccine

Long-term control of blood pressure is important for primary and secondary prevention of ischemic stroke. Diuretics, β-blocker, calcium channel blocker, angiotensin-converting enzyme inhibitors, and angiotensin receptor blocker are clinically used as antihypertensive agents. In addition to lowering the BP, inhibition of renin–angiotensin (Ang) system (RAS) by Ang-converting enzyme inhibitors (ACE-Is) or Ang II receptor blockers (ARBs) is effective in the primary (Ravenni et al. 2011) and secondary prevention of ischemic stroke (Lee et al. 2012) because Ang II increases the reactive oxygen species production and oxidative stress in ischemic brain (Horiuchi and Mogi 2011; Saavedra 2012). Independent of systemic RAS, Ang II is locally produced in brain from tissue angiotensinogen by renin and ACE (Hosomi et al. 2013). In MCAO, expression of AT1R mRNA was increased at 24 h after middle cerebral artery occlusion (Wu et al. 2013) and angiotensinogen mRNA was increased at 1–2 h followed by return to baseline level at 6 h (Fu et al. 2011). AT1R mRNA is expressed in neurons and NADPH oxidase2 is involved in the AT1R-reactive oxygen species axis in neurons (Garrido and Griendling 2009; Wang et al. 2013). The neuroprotective and antioxidative stress effects were observed in

ARBs in the cultured neurons via inhibition of such signals (Benicky et al. 2011; Pang et al. 2012; Zhao et al. 2015).

The long-term inhibition of RAS with vaccine will be a promising strategy for preventing stroke onset and will also protect the brain from ischemic injury. Because the peptide vaccine targeting Ang II, namely the "Ang II vaccine" or "Ang II DNA vaccine," was known to produce sufficient antibody against Ang II to exert an antihypertensive effect in animal models (Tissot et al. 2008; Nakagami et al. 2013a; Koriyama et al. 2015) and human clinical trials (Tissot et al. 2008), which included nonstroke patients, it will at least be effective in terms of lowering blood pressure. Differing from the antithrombotic vaccine, which acts on platelets in blood, the ability of the antibody induced against Ang II to penetrate into brain parenchyma beyond the blood–brain barrier (BBB) is an important factor because the antibodies act on inflammatory cells or neurons in the ischemic cerebral parenchyma. Nevertheless, the antibody could hardly penetrate the BBB in normal state. For example, a previous paper reported that 0.11% of intravenously injected antibodies against amyloid β protein crossed the BBB within 1 h in normal mice (Banks et al. 2002). However, in ischemic stroke, breakdown in the BBB was reported to begin 2 h after the antibody injection in rodent models (Gasche et al. 2001), and we examined whether the antibodies produced upon vaccination could penetrate the brain parenchyma in ischemic brain (Wakayama et al. 2017). In vaccinated rats, there were variations of antibody titer for Ang II in serum, and rats indicating higher titer revealed lower infarct volume (Fig. 3.3a). When the rats were divided into a V_H group with injection of more than 6000 antibody titer and a V_L group with less than 6000 antibody titer, those in V_H group presented significant reduction in the infarct volume (Fig. 3.3b). Moreover, the Ang II antibody titer was increased in the brain parenchyma from 12 h after MCAO (Fig. 3.3c, d). These antibodies against Ang II could penetrate the ischemic brain and sufficient antibody titers were required to achieve the cerebroprotective effect. Because the vaccinated rats showed reduction in Fluoro-Jade B–positive cells, spectrin fragmentation, 4-hydroxynonenal-positive cells, and Nox2 mRNA expression, the antibodies produced against Ang II penetrated into the brain parenchyma beyond destructed BBB and protected the brain via inhibition of oxidative stress and neuroprotection (Wakayama et al. 2017). Because a dynamic contrast-enhanced MRI revealed that patients examined at 1.3–90.7 h after stroke onset showed increased permeability of BBB (Merali et al. 2017), antibodies produced by vaccine in the future clinical trial will penetrate the ischemic brain parenchyma.

The important side effect of antihypertension vaccine is the excessive lowering of BP because very low–normal (<120 mm Hg) mean SBP level is a risk factor for ischemic stroke (Ovbiagele et al. 2011); however, we speculate that vaccine targeting Ang II is safe because our previous studies on Ang II vaccine reported no BP-lowering effects in normotensive mice (Nakagami et al. 2013b) and rats (Wakayama et al. 2017). Another concern is that it takes several weeks to achieve sufficient antibody titer while using the Ang II vaccine as the secondary preventive measure in ischemic stroke. Similar to the antithrombotic vaccine, combination of vaccine with ARB or ACE-I is necessary until the antibody titer is considerably high

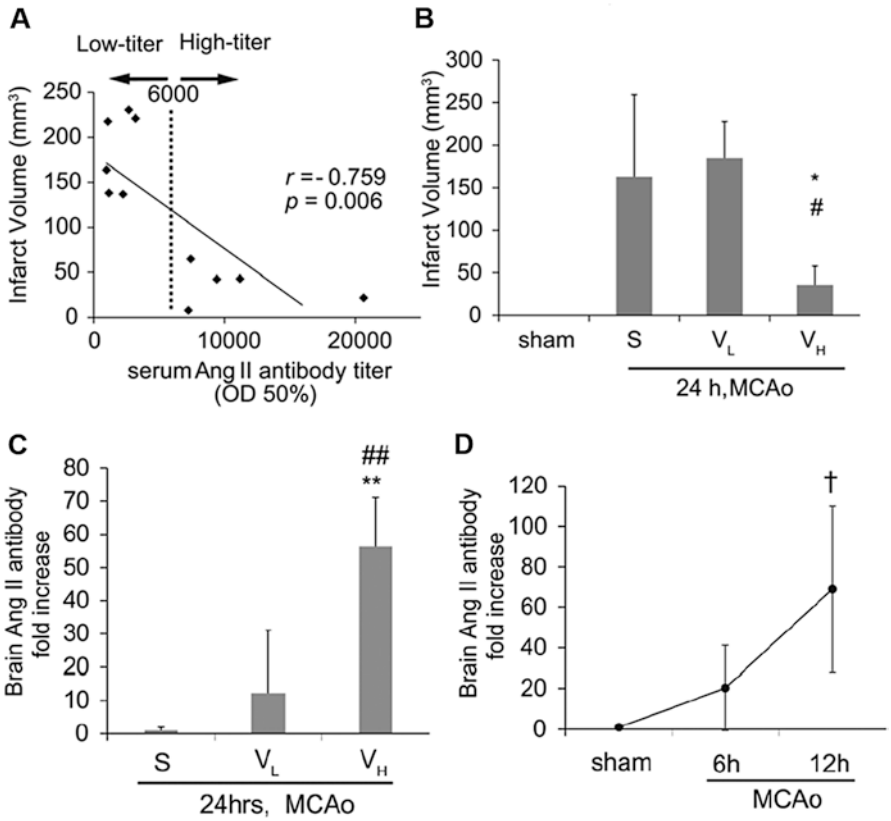

Fig. 3.3 Effect of Ang II vaccine for ischemic stroke (cited from (Wakayama et al. 2017)) (**a**) Higher antibody titer revealed lower infarct volume. (**b**) Low infarct volume was presented in V_H rats whose serum anti-Ang II antibody titer was OD 50% ≥ 6000. $*P < 0.05$ versus S, $\#P < 0.05$ versus VL. Each group included $n = 5$ (sham), $n = 5$ (VH), $n = 6$ (VL), or $n = 8$ (S) in B. (**c**) V_H rats revealed a significant increase in anti-Ang II antibody in ischemic brain tissue. (**d**) Anti-Ang II antibody in ischemic brain had increased as early as 12 h after pMCAO in VH rats. $**P < 0.01$ versus VL, $\#\#P < 0.01$ versus S, $\dagger P < 0.05$ versus VH (sham). Each group included $n = 7$ (S), $n = 7$ (V_H), $n = 10$ (V_L) in C and $n = 5$ (V_H [sham]), $n = 7$ (V_H [6 h]), $n = 6$ (V_H [12 h]) in D

to present its efficacy. Therefore, the safety about this combination should be investigated. Moreover, the variation of antibody titers should be resolved as low antibody titers did not reveal cerebroprotective effects. As it is ideal to get sufficient antibody titers constantly, further studies on the amount of vaccine or adjuvant are necessary.

Thus, antihypertension vaccine has certain concerns, but the long-lasting effect is promising for the improvement of adherence of poststroke patients. Further studies about the safety and combination with existing drugs will make it possible to use the antihypertension vaccine in clinical trials.

Declaration of Interest The Department of Health Development and Medicine is financially supported by AnGes, DAICEL, and FunPep. The Department of Advanced Clinical Science and Therapeutics is financially supported by AnGes.

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Chapter 4
Immunotherapy for Obesity

Tatsuhiko Azegami and Hiroshi Itoh

Abstract Obesity prevalence continues to increase in both adults and children worldwide and greatly contributes to increased morbidity and mortality. Although there are some anti-obesity drugs globally available for clinical use, their inadequate effectiveness coupled with safety concerns sometimes discourage the widespread use of anti-obesity medication. Because of its prolonged therapeutic effect and low frequency of administration, a therapeutic vaccine may be an attractive strategy for the prevention and treatment of obesity. Over the last two decades, several attempts have been made to develop vaccines for the control of obesity. Animal studies have shown that vaccines targeting ghrelin, glucose-dependent insulinotropic polypeptide, adipocytes, somatostatin, and adenovirus 36 successfully led to a reduction in weight gain without serious adverse effects. This chapter provides an overview of recent progress toward a therapeutic vaccine against obesity.

Keywords Obesity · Ghrelin · Glucose-dependent insulinotropic polypeptide · Adipocyte · Somatostatin · Adenovirus 36

4.1 Introduction

Obesity is defined as a medical condition of abnormal or excessive fat accumulation that may impair health. The body mass index (BMI) is an estimate of body fat based on height and weight and is used clinically to diagnose obesity. For adults, overweight and obesity are defined as BMI $\geq$ 25 kg/m^2 and $\geq$ 30 kg/m^2, respectively.

T. Azegami (✉)
Health Center, Keio University, Yokohama, Japan

Department of Internal Medicine, School of Medicine, Keio University, Tokyo, Japan
e-mail: t.azegami-1114@z2.keio.jp

H. Itoh
Department of Internal Medicine, School of Medicine, Keio University, Tokyo, Japan

H. Nakagami (ed.), *Therapeutic Vaccines as Novel Immunotherapy*,
https://doi.org/10.1007/978-981-32-9628-2_4

According to the World Health Organization (WHO) fact sheets (16 February 2018, URL: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight), in 2016, more than 1.9 billion (39%) adults were overweight and 650 million (13%) were obese worldwide. Among children and adolescents in particular, the prevalence of overweight has increased markedly from 4% in 1975 to over 18% in 2016 and that of obesity has also risen explosively from less than 1% to 7%.

Obesity is associated with high all-cause mortality (Flegal et al. 2013) and increased risk of cardiovascular diseases (Wilson et al. 2002), heart failure (Kenchaiah et al. 2002), end-stage renal disease (Vivante et al. 2012), and some cancers (Renehan et al. 2008). Due to its rising prevalence and increased comorbidity burden, obesity is predicted to cost the US healthcare system $48–$66 billion per year by 2030 (Wang et al. 2011). To combat the economic burden of obesity and its comorbidities, innovative strategies to prevent or treat obesity are needed.

Obesity is a chronic disease that involves interactions between environmental and genetic factors. Because environmental factors that lead to an imbalance between energy intake and expenditure are the main driver for obesity, comprehensive lifestyle modifications such as reduced energy intake and increased physical activity are generally the first approach for weight reduction (Bray et al. 2016). In fact, a comprehensive program of lifestyle modification can produce a 7–10% reduction in body weight at 1 year or more (Wadden et al. 2012). Pharmacotherapy should be considered when patients have difficulty achieving or maintaining sufficient weight reduction through lifestyle modification alone.

Six main drugs are currently approved as anti-obesity medications by the Food and Drug Administration (FDA): phentermine (sympathomimetic amine), orlistat (lipase inhibitor), phentermine/topiramate (sympathomimetic amine and an antiepileptic drug), lorcaserin (5-HT_{2c} receptor agonist), naltrexone/bupropion (opioid antagonist and an aminoketone antidepressant), and liraglutide (glucagon-like peptide-1 [GLP1] receptor agonist) (Srivastava and Apovian 2018). Orlistat decreases the absorption of dietary fat, whereas the other drugs suppress appetite and increase satiety through the stimulation of the central nervous system (Fig. 4.1) (Srivastava and Apovian 2018). However, physicians are often reluctant to prescribe weight-loss medications due to patient dissatisfaction with the amount of weight reduction, lingering concerns about drug safety, and weight regain after discontinuation of the medication (Heymsfield and Wadden 2017).

Therapeutic vaccines have the potential to contribute to obesity therapy because of their prolonged therapeutic effect and low frequency of administration. Over the last two decades, numerous attempts have been made to develop vaccines for the prevention and treatment of obesity (Table 4.1). In this chapter, we will introduce and summarize some of the candidates for innovative therapeutic vaccines against obesity.

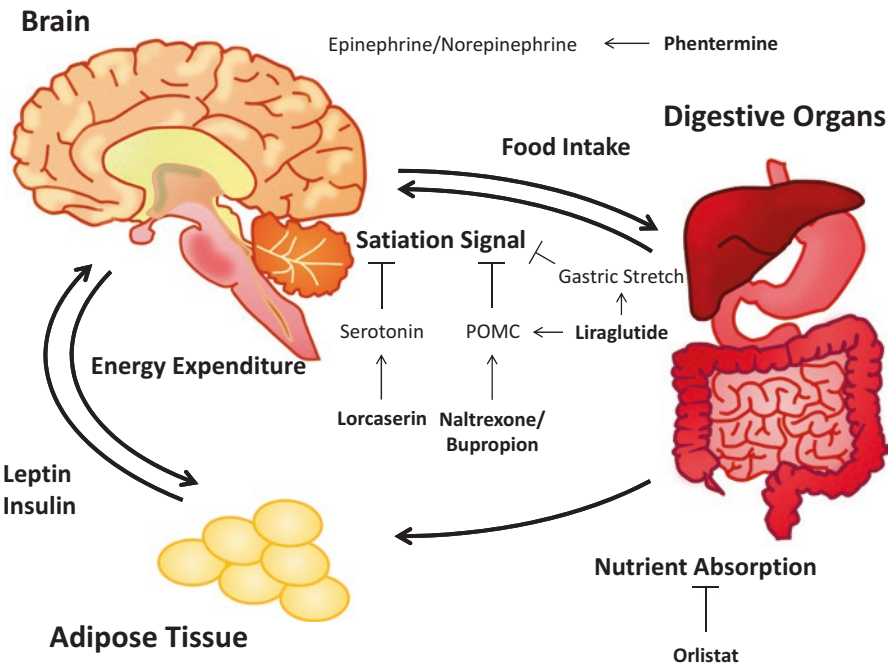

Fig. 4.1 Schematic representation of the pathogenesis of obesity and the mechanisms of action of anti-obesity drugs
POMC: pro-opiomelanocortin

4.2 Vaccination Targets Against Obesity

4.2.1 Ghrelin

Ghrelin is a 28 amino-acid peptide hormone that possesses a unique *O*-acylation at the Ser^3 residue and has been identified as a ligand for growth-hormone secretagogue receptor (GHSR) (Kojima et al. 1999). Ghrelin is the only known circulating peripheral hormone that promotes body weight gain by stimulating food intake and decreasing energy expenditure (Nakazato et al. 2001; Tschop et al. 2000; Wortley et al. 2004). Ghrelin is mainly produced in the gastric X/A-like cells, but is also expressed in the small intestine, brain, pancreas, and other peripheral organs (Ghelardoni et al. 2006). Peripheral ghrelin modulates the nucleus tractus solitarius via the vagus nerve, which results in an increase in noradrenaline in the arcuate nucleus of the hypothalamus and appetite stimulation (Date et al. 2006). The orexigenic action of ghrelin requires *O*-acylation at Ser^3 with octanoate, an eight-carbon fatty acid, and unacylated ghrelin (also called des-acyl ghrelin) has opposite effects from those of ghrelin (i.e., acylated ghrelin) on body weight in that it decreases

Table 4.1 Developmental therapeutic vaccines against obesity

Vaccine target	Antigen/ adjuvant	Immunization protocol	Effect	Animals/ humans	Reference
Ghrelin	Ghr1-KLH, Ghr2-KLH, Ghr3-KLH/ Ribi, and alum adjuvant	Five doses of intraperitoneal immunization with 250 μg of antigen	No change in food intake 20% reduction in weight gain (Ghr1, Ghr3)	Male Wister rat	Zorrilla et al. (2006)
	N-terminal ghrelin (1–10)-BSA/ FIA & DEAE	Three doses of subcutaneous immunization with 50 μg of antigen	15% reduction in food intake 10% reduction in weight gain	Male/female piglet	Vizcarra et al. (2007)
	Ghrelin-NS1	Three doses of intraperitoneal immunization with 75 μg of antigen	No change in food intake increase in energy expenditure No change in weight gain	DIO male C57BL6/J mouse	Andrade et al. (2013)
	Ghrelin-PspA/ c-di-GMP	Five doses of intranasal immunization with 5 μg of antigen	No change in food intake 7% increase in energy expenditure 7% reduction in weight gain (DIO)	Male C57BL6/J mouse (DIO) and *ob/ob* mouse	Azegami et al. (2017)
GIP	GIP-QB	Four doses of subcutaneous immunization with 100 μg of antigen	No change in food intake Increase in energy expenditure 35% reduction in weight gain	DIO female C57BL6/J mouse	Fulurija et al. (2008)
Adipocyte	Mouse adipocyte	Four doses of intraperitoneal immunization with 10^6 adipocytes	About 50% reduction in weight gain	Male/female Sprague Dawley rat	Lai et al. (2010)
	Pig adipose tissue	Daily oral doses for 3 months	No change in body weight 7.6% reduction in waist size 25.9% increase in HDL-C	Male/female human adult	Bourinbaiar and Jirathitikal (2010)

(continued)

Table 4.1 (continued)

Vaccine target	Antigen/ adjuvant	Immunization protocol	Effect	Animals/ humans	Reference
Somatostatin	Somatostatin-CAT/JH17 or JH18	One dose of intraperitoneal immunization with 500 μg of antigen	12–13% reduction in body weight No change in food intake	DIO male C57BL6/J mouse	Haffer (2012)
Adenovirus 36	Inactivated Ad36/FCA or FIA	Two doses of intraperitoneal immunization with 5 μg of antigen	No change in food intake 17% reduction in weight gain 20% reduction in epididymal fat	Ad36-infected C57BL6/J mouse	Na and Nam 2014

GIP glucose-dependent insulinotropic polypeptide, *KLH* keyhole limpet hemocyanin, *BSA* bovine serum albumin, *FIA* Freund's incomplete adjuvant, *DEAE* diethylaminoethyl-dextran, *NS1* non-structural protein 1, *PspA* pneumococcal surface protein A, *CAT* chloramphenicol acetyl transferase, *FCA* Freund's complete adjuvant, *DIO* diet-induced obesity

appetite and body weight (Asakawa et al. 2005). Ghrelin *O*-acyltransferase (GOAT) is the enzyme responsible for the octanylation of ghrelin (Yang et al. 2008).

Although ghrelin would appear to be an attractive target for the development of a drug to treat or prevent obesity, as of 2018, there is no clinically available anti-obesity drug that targets ghrelin function, such as a ghrelin inhibitor, GHSR antagonist, or GOAT inhibitor. Animal experiments have suggested that inhibition of ghrelin function is effective against obesity. A few reports indicate that the genetic deletion of ghrelin does not alter food intake and body weight (Wortley et al. 2004), but ghrelin-deficient mice have increased energy expenditure and locomotor activity and are protected from diet-induced obesity after early exposure to a high-fat diet from 3 weeks after weaning (Kushnir et al. 2012). Genetic deletion of the ghrelin receptor GHSR also suppresses weight gain in mice fed a high-fat diet (Zigman et al. 2005). In terms of the drug development, GOAT inhibitors and GHSR antagonists have the potential to attenuate diet-induced obesity (Ambuhl et al. 2007; Maurer et al. 2005); however, none have been approved for clinical use.

The first attempt to prevent weight gain by using a therapeutic anti-ghrelin vaccine was reported in 2006 (Zorrilla et al. 2006). Zorrilla et al. synthesized three different ghrelin antigens (Ghr1, Ghr2, and Ghr3) as candidate vaccine antigens and coupled them to the carrier protein keyhole limpet hemocyanin (KLH) (Zorrilla et al. 2006). Ghr1 spanned N-terminal residues 1–10 and was butanoylated at Ser^3, Ghr2 comprised C-terminal residues 13–28, and Ghr3 spanned the whole ghrelin analog (1–28) and contained Ser^3-(butanoyl). Rats were immunized with these vaccine candidates conjugated with Ribi adjuvant, an oil-in-water emulsion containing monophosphoryl lipid A, on days 0, 21, and 35, and subsequently immunized with the vaccines conjugated with alum adjuvant on days 56 and 84. Rats that received five immunizations with Ghr1-KLH, Ghr2-KLH, or Ghr3-KLH developed antigen-specific antibodies that did not cross-react with the other antigens. Rats immunized

with Ghr1-KLH or Ghr3-KLH developed an antibody that possessed good plasma binding affinity for acylated ghrelin and gained less weight than non-immunized rats without changes in their food consumption. In contrast, vaccination with Ghr2-KLH did not affect weight gain.

Vizcarra et al. also reported on the effectiveness of a peptide vaccine composed of the N-terminal region of ghrelin (Vizcarra et al. 2007). Pigs subcutaneously immunized three times with a 20-day interval with a vaccine comprising the N-terminal residues (1–10) of porcine ghrelin coupled with bovine serum albumin (BSA) and conjugated with Freund's incomplete adjuvant and diethylaminoethyl-dextran showed a decrease in both daily food intake (15% less than control) and weight gain (10% less than control) (Vizcarra et al. 2007).

Recently, advances in biomedical technology and nanotechnology, such as virus-like particles (VLPs) and nanometer-sized polymer hydrogel (nanogel), have been applied to the development of anti-ghrelin vaccines. VLPs are formed by structural viral proteins that self-assemble and display antigenic epitopes in the correct conformation and in a highly repetitive manner (Kushnir et al. 2012). VLP-based vaccines targeting non-communicable diseases such as nicotine dependence and hypertension were found to be safe and well-tolerated in human clinical trials and to successfully induce antigen-specific antibodies (Ambuhl et al. 2007; Maurer et al. 2005). Andrade et al. conjugated VLPs consisting of tubules of non-structural protein (NS) 1 of Bluetongue virus to ghrelin peptide and then intraperitoneally immunized mice with 75 μg of the ghrelin-NS1 immunoconjugate three times with 2-week intervals (Andrade et al. 2013). The immunized mice raised ghrelin-specific serum IgG antibodies and showed increased energy expenditure and decreased food intake, but no change in body weight (Andrade et al. 2013). The lack of weight reduction despite decreased food intake and increased energy expenditure after vaccination with ghrelin-NS1 may be partially explained by the relatively short follow-up period or the possible activation of compensatory mechanisms of energy homeostasis or both. Interestingly, feedback responses leading to increased ghrelin expression in the stomach did not occur after immunization. Ghrelin-NS1 vaccination also promoted the formation of circulating immune complexes of ghrelin on anti-ghrelin antibodies and resulted in increased fasting plasma ghrelin concentrations (Andrade et al. 2013).

We recently developed a new anti-ghrelin therapeutic vaccine that can be administered intranasally (Azegami et al. 2017). Intranasal immunization offers distinct advantages over other injectable delivery methods including decreased cost, less psychological and physiological stress, and no risk of localized skin adverse events (Lamichhane et al. 2014). However, nasal administration of antigen alone often fails to induce sufficient antigen-specific mucosal and systemic immune responses (Azegami et al. 2018). Application of a nanogel, which is a promising vaccine-antigen delivery vehicle, has great potential in vaccine development because nanogels can incorporate various proteins through hydrophobic interactions, which prevents the irreversible aggregation of the incorporated proteins, and subsequently allows their release in their native form (Azegami et al. 2018). Among nanogels, the cationic type of cholesteryl-group-bearing pullulan (cCHP) nanogel is suitable as a

intranasal vaccine-delivery system because the cationic property of cCHP nanogels allows efficient adhesion of the nanogels to the negatively charged nasal epithelial layer, leading to effective and continuous delivery of the vaccine antigen to the dendritic cells beneath the nasal epithelial cells (Nochi et al. 2010). We created a new vaccine antigen, ghrelin-PspA (pneumococcal surface protein A), which is a recombinant fusion protein incorporating three repeats of mouse whole ghrelin and PspA as a carrier protein (Azegami et al. 2017). Intranasal immunization of mice with the ghrelin-PspA vaccine, which comprised 5 μg of antigen and 10 μg of cyclic di-GMP adjuvant within a cCHP nanogel, on five occasions at 1-week intervals induced ghrelin-specific serum IgG antibodies and attenuated body weight gain (7% less than control) in diet-induced obese mice (Azegami et al. 2017). This anti-obesity effect was caused by a decrease in both visceral and subcutaneous fat accumulation and an increase in energy expenditure that was partially due to the increased expression of mitochondrial uncoupling protein 1 in brown adipose tissue (Azegami et al. 2017). In addition, intranasal vaccination with ghrelin-PspA decreased the body weight of genetically obese *ob/ob* mice (control +4.7 g vs. ghrelin-PspA −0.9 g, weight change between pre- and 1 week post-vaccination). The ghrelin-PspA vaccine also promoted the formation of circulating immune complexes of ghrelin on anti-ghrelin antibodies and prolonged the circulating half-life of ghrelin, resulting in increased fasting plasma ghrelin concentrations, similar to the ghrelin-NS1 vaccine (Azegami et al. 2017).

4.3 Glucose-Dependent Insulinotropic Polypeptide

Incretin hormone is secreted from enteroendocrine cells in the intestinal epithelium and regulates glucose metabolism. In humans, GIP (glucose-dependent insulinotropic polypeptide) and GLP-1 are the two main incretin hormones and stimulate pancreatic insulin secretion in a glucose-dependent manner (Sadry and Drucker 2013). In addition to its insulinotropic action, GIP also promotes adipose tissue accretion. GIP receptor-deficient mice exhibit increased energy expenditure and are resistant to diet-induced obesity (Miyawaki et al. 2002). Administration of a GIP receptor antagonist also attenuates diet-induced obesity and improves glucose metabolism in mice (McClean et al. 2007); accordingly, GIP is a strong target candidate for anti-obesity treatment.

To induce a humoral immune response against a self-derived peptide (i.e., GIP), Fulurija et al. coupled the N-terminal residues (1–15) of GIP to the surface of bacteriophage Qβ VLPs (Fulurija et al. 2008). Four subcutaneous immunizations with 100 μg of VLP-based GIP vaccine with 2-week intervals elicited antigen-specific serum IgG antibodies that bound to circulating GIP and reduced diet-induced body weight gain in mice (35% less weight gain than control) without inducing an autoinflammatory reaction in the intestine or disturbing glucose homeostasis (Fulurija et al. 2008). This anti-obesity effect of the GIP vaccine was derived from suppres-

sion of fat accumulation and caused by an increase in energy expenditure due to a high basal metabolic rate (Fulurija et al. 2008).

4.4 Adipocytes

Obesity results from excessive accumulation of adipose tissue, and adipose tissue dysfunction causes metabolic complications in the obese. Therefore, treatment that directly targets adipose tissues is an attractive strategy to overcome obesity and its metabolic comorbidities.

Two unique approaches to the development of an adipocyte-based anti-obesity vaccine were reported in 2010. Lai et al. examined the efficacy of mouse 3T3-L1 adipocytes as a xenogeneic adipocyte vaccine in rats (Lai et al. 2010). Sprague Dawley rats were intraperitoneally immunized with 10^6 mouse 3T3-L1 adipocytes weekly for 4 weeks and fed a high calorie diet after the final immunization. Xenogeneic adipocyte vaccination dramatically decreased weight gain and induced adipocyte apoptosis (Lai et al. 2010).

Bourinbaiar and Jirathitikal conducted a clinical trial to evaluate the efficacy and safety of antigenic adipose tissue on body weight and lipid metabolism (Bourinbaiar and Jirathitikal 2010). In this clinical study, 9 females and 4 males, aged between 22 and 79, received oral tablets containing pig adipose tissue for 3 months. The small sample size and short observation period in this single-arm experiment were clear limitations, and unfortunately it is unclear whether oral vaccination with xenogeneic adipocytes induced a humoral immune reaction against adipose tissue in the immunized subjects. However, oral adipocyte vaccine successfully reduced waist size (−7.6%) and increased serum high-density lipoprotein cholesterol levels (+25.9%) without causing any adverse events in the human subjects, but it did not change body weight, or low-density lipoprotein cholesterol and triglyceride levels.

4.5 Somatostatin

Growth hormone (GH) deficiency is known to cause an increase in fat accumulation, whereas GH replacement decreases body fat in patients with adult-onset GH deficiency (Baum et al. 1996). Moreover, low-dose GH treatment decreases body fat in obese adults who are not GH deficient (Kim et al. 1999). Although GH therapy may be a promising strategy for the attenuation of obesity, its clinical application is limited by its very short half-life, necessitating a daily subcutaneous injection (Faria et al. 1989). As an alternative strategy, the sustained inhibition of somatostatin, an endogenous suppressor of pituitary GH secretion, may be a therapeutic option for GH-mediated anti-obesity treatment.

In 2012, a chimeric polypeptide consisting of somatostatin and the carrier protein chloramphenicol acetyltransferase was developed as an anti-somatostatin vac-

cine for the inhibition of somatostatin effects (Haffer 2012). To enhance its immunogenicity, chimeric somatostatin antigen was mixed with either JH17 or JH18 adjuvant as well as squalene, Tween 80, and Span 85 with or without tragacanthin and arabinogalactan. Intraperitoneal immunization with the somatostatin vaccine containing 500 μg of antigen elicited chimeric antigen protein (not somatostatin alone)-specific serum IgG antibodies and slightly increased the levels of insulin-like growth factor 1, which is secreted by the liver upon GH stimulation. Surprisingly, a single dose of the somatostatin vaccine immediately caused weight reduction (12.2% and 13.1% for the JH17 and JH18 adjuvants, respectively) compared with PBS controls without a change in food intake in diet-induced obese mice at only 4 days post-immunization.

4.6 Adenovirus 36

Obesity is a multifactorial disease caused by environmental and genetic factors. Among the environmental factors, although food and physical activity play the biggest role in the development of obesity, a link between virus infections such as adenovirus 36 (Ad36) and obesity has also been shown through animal and human studies (Dhurandhar et al. 2002; Lyons et al. 1982). In one human study, 30% of obese subjects were positive for serum Ad36 antibody (i.e., evidence of prior Ad36 infection) compared with 11% of non-obese subjects (Atkinson et al. 2005). Inoculation of Ad36 directly promotes weight gain and fat accumulation in non-human primates (Dhurandhar et al. 2002). Although the pathogenic mechanism responsible for Ad36-induced obesity remains to be fully elucidated, Ad36 enhances the differentiation of pre-adipocytes via E4 open reading frame-1 gene signaling, reduces leptin production, and increases glucose uptake by adipocytes (Rogers et al. 2008; Vangipuram et al. 2004; Vangipuram et al. 2007).

Na and Nam chose Ad36 as the therapeutic target of their novel anti-obesity vaccine (Na and Nam 2014). They used UV-irradiated inactive Ad36 as a vaccine antigen. Two doses of 5 μg of inactivated Ad36 in Freund's adjuvant were intraperitoneally injected into mice with 2-week intervals. This regimen attenuated body weight gain, fat accumulation, and adipose tissue inflammation in the Ad36-inoculated mice without altering food consumption (Na and Nam 2014).

4.7 Future Perspectives

Obesity is one of the biggest health problems in the world. To overcome the global obesity epidemic, the development of public education programs that raise awareness of obesity and its consequent health risks is essential. In addition, obesity must be tackled at the individual level, encouraging comprehensive lifestyle modification. Because of their prolonged therapeutic effects and low frequency of adminis-

tration, therapeutic vaccines may have potential as an approach to the prevention and treatment of obesity. Induction of the humoral immune response (i.e., B cell activation) is essential to elicit antigen-specific antibodies to neutralize endogenous targets that promote obesity. To effectively induce a humoral immune response against an endogenous self-derived target, various adjuvants are widely used. However, they may also trigger undesired autoimmune reactions; therefore, careful consideration of not only the therapeutic effect but also the risk for autoimmune reactions must be applied to a therapeutic vaccine for clinical use. To overcome this concern, recent advances in nanotechnology and biotechnology such as VLPs and other delivery vehicles will assist in the development of innovative vaccines.

Acknowledgments This work was supported in part by the Mochida Memorial Foundation for Medical and Pharmaceutical Research.

Conflict of Interest The authors have no conflicts of interest to declare.

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Chapter 5
Immunotherapy for Spondyloarthritis (SpA)

Jiao Sun and Hiroki Hayashi

Abstract Spondyloarthritis (SpA) is an umbrella term for chronic inflammatory conditions that affect the joints and entheses (the region of attachment between ligaments or tendons and bones) at the sacroiliac region and spine, negatively impacting the patient's daily life. Because human leukocyte antigen (HLA)-B27, a class I surface antigen encoded in the major histocompatibility complex (MHC), was identified as a strong genetic risk factor, several major theories for the pathological mechanisms have been proposed based on in vitro and translational studies. Additionally, emerging lines of evidence over the last decade suggest that the IL-23/IL-17 axis has a critical role (roles) in the pathology of SpA. Recently, molecular immunotherapy using antibodies has been studied and developed as a therapeutic option to treat SpA. This chapter describes antibody-based immunotherapies targeting arthritogenic inflammatory pathways based on proposed HLA-B27-related mechanisms.

Keywords HLA-B27 · Autoimmune disorder · Endoplasmic reticulum (ER) · Unfolded protein reaction (UPR) · IL-17A · IL-23 · TNF-α

5.1 Introduction

Spondyloarthritis (SpA) is a term for inflammatory disorders of the spinal and sacroiliac joints, which affects 0.5–1% of the population worldwide (Braun and Sieper 2007; Colbert et al. 2010). SpA is believed to be initially triggered by inflammation via an autoimmune-mediated mechanism in tendons and enthesis in the sacroiliac joints along with the spine, leading to ankylosis with bone erosion followed by new bone formation, which is one of the main hallmarks of ankylosing spondylitis (AS),

J. Sun · H. Hayashi (✉)
Department of Health Development and Medicine, School of Medicine, Osaka University, Suita, Osaka, Japan
e-mail: hayashih@cgt.med.osaka-u.ac.jp

H. Nakagami (ed.), *Therapeutic Vaccines as Novel Immunotherapy*,
https://doi.org/10.1007/978-981-32-9628-2_5

a prototype of the disease (Sherlock et al. 2012; Zochling and Smith 2010). Clinically, SpA includes AS, the most common form, as well as psoriatic arthritis (PsA), reactive arthritis (ReA), enteropathic arthritis (EA), and undifferentiated SpA (uSpA) (Inman 2006).

Although the precise pathological mechanism by which articular inflammation is triggered is unclear, various risk factors have been reported since this condition was first recognized in a group of patients with seronegative arthritis in the 1970s. One of these factors is major histocompatibility complex class 1 antigen B27 (HLA-B27), which is a strong genetic risk factor. Approximately, 95% of AS patients carry HLA-B27 (Brewerton et al. 1973; Schlosstein et al. 1973). Indeed, transgenic rats with HLA-B27 and human β_2-microgloblin (hβ_2m) exhibited inflammatory diseases in peripheral and axial joints as well as other organs, such as the gut and heart (Hammer et al. 1990). Recently, the dysregulated IL-23-IL-17 pathway and related inflammatory cytokines (IFN-γ, TNF-α) have been identified in an experimental animal model and translational studies (Gravallese and Schett 2018; Raychaudhuri and Raychaudhuri 2016; Smith and Colbert 2014).

Because molecular pathways involved in the pathophysiology of SpA have been defined, many drugs targeting these molecules/pathways have been developed or are currently being developed in preclinical and clinical studies. This chapter will focus on therapeutic approaches for SpA, especially immunotherapy.

5.2 Etiology (Proposed Mechanisms of SpA)

As described above, the molecular mechanism of SpA is still unclear; however, HLA-B27 has been reported to have a strong relationship with spondyloarthropathy, especially AS (Brewerton et al. 1973; Hammer et al. 1990; Schlosstein et al. 1973). Many studies using animal models (HLA-B27 and hβ_2m transgenic rats) and translational studies led us to propose three possible HLA-B27-mediated pathogenic theories to explain the SpA phenotype (Colbert et al. 2010; Powis and Colbert 2016).

First, HLA-B27 was shown to present peptides derived from intracellular proteins to normally induce CD8 T cell responses (Bowness 2002). However, HLA-B27 was suggested to trigger arthritogenic CD8 T cell reactions by expressing a peptide of infected microorganisms with a combination of self-antigens at the cell surface to produce an autoimmune reaction, which can be observed in patients who developed reactive arthritis (“presentation of arthritogenic peptides”) (Benjamin and Parham 1990; Benjamin et al. 1991; Hermann et al. 1993) (Fig. 5.1).

Second, HLA-B27 was shown to form homodimers (Allen et al. 1999), and the HLA-B27 molecule misfolds in the endoplasmic reticulum (ER) to induce the unfolded protein response (UPR) or ER-stress response, which results in augmented IL-23 production, as shown in vitro using macrophages or dendric cells and in

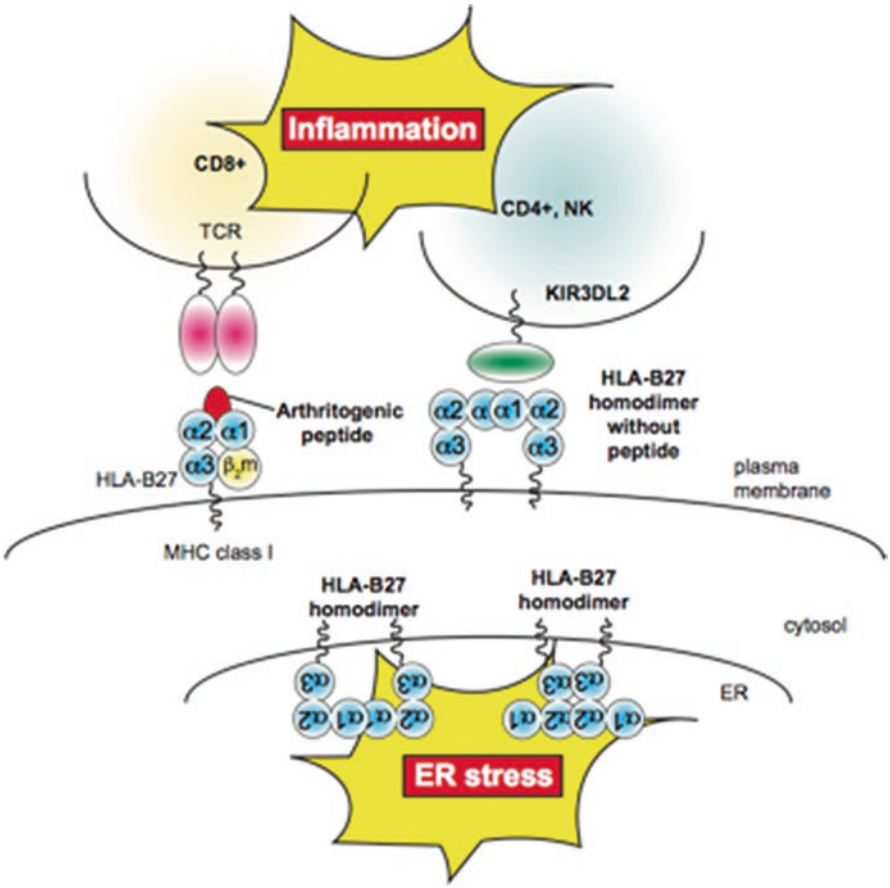

Fig. 5.1 Schematic diagram of HLA-B27-mediated arthritogenic theories
Three possible theories have been proposed. First, the presentation of "arthritogenic peptides" to CD8+ T cells. Second, induction of "ER stress" by abnormal dimer formation of HLA-B27 (UPR) in the ER. The HLA-B27 homodimer is mediated by Cys67 or Cys164. Third, activation of immune cells by "HLA-B27 homodimer without peptides at cell surface" through T cell receptors (e.g., KIR3DL2). These theories induce proinflammatory mediators. Abbreviations: *HLA* human leukocyte antigen, *ER* endoplasmic reticulum, *UPR* unfolded protein responses, *β_2m* β_2-microglobulin, *KIR3DL2* killer cell immunoglobulin like-receptor, 3 Ig domains and long cytoplasmic tail 2

animal studies ("UPR and ER stress") (DeLay et al. 2009; Goodall et al. 2010; Mear et al. 1999; Turner et al. 2007) (Fig. 5.1).

Third, HLA-B27 homodimers mediated by Cys67 without β_2-microglobulin (β_2m-free dimer) or without the heavy chain (HC) have been reported to be expressed at the cell surface (Allen et al. 1999; Bird et al. 2003) and induce arthritogenic immune activation (e.g., the Th17 axis) through binding with innate immune

receptors, such as the killer cell immunoglobulin-like receptor, 3 Ig domains and long cytoplasmic tail 1(KIR3DL1), KIR3DL2, and leukocyte immunoglobulin-like receptor subfamily B member 2 (LILIRB2) and paired immunoglobulin receptors (PIR) on T, NK, and myeloid cells ("cell-surface HLA-B27 abnormal formation") (Bowness et al. 2011; Chan et al. 2005; Giles et al. 2012; Kollnberger et al. 2002, 2004; Wong-Baeza et al. 2013) (Fig. 5.1).

In addition to those theories, AS was shown to be strongly correlated with aminopeptidases, including endoplasmic reticulum amino peptidase 1 (ERAP1) (Evans et al. 2011), ERAP2, leucyl-cystinyl aminopeptidase (LNPEP), and puromycin-sensitive aminopeptidase (PSA/NPEPPS) (International Genetics of Ankylosing Spondylitis et al. 2013), suggesting that the pathophysiology of AS might have a strong relationship with peptide handling processes. Interestingly, other recent studies using animal models and genome-wide association studies (GWAS) have identified the relationship between HLA-B27 and the gut microbiome (Lin et al. 2014; Van Praet et al. 2013). Moreover, IL-23/IL-23R signaling in RAR-related orphan receptor γt^{+} $CD3^{+}CD4^{-}CD8^{-}$ $Sca1^{+}$ T cells residing in enthesis was sufficient to trigger the SpA-like phenotype in an animal model (Sherlock et al. 2012), which was similar to the conditions in patients with AS. Mechanical stress was previously shown to be a risk factor for SpA pathology (Jacques and McGonagle 2014), confirming that environmental factors in lifestyle, including occupations, affect the manifestation of SpA (Ramiro et al. 2015).

Thus, HLA-B27, a strong genetic risk factor, and environmental factors also have a critical role in the pathophysiology of SpA by dysregulating immune responses with arthritogenic inflammation.

5.3 Immunotherapy for SpA

Since SpA disease was first reported, immune-activated inflammation has been shown to contribute to the phenotypic development of SpA. In the past decade, in vitro studies and clinical studies of arthritogenic-triggered inflammation, including proinflammatory cytokines, especially TNF-α, IL-23, and IL-17, were used to identify therapeutic targets (Gravallese and Schett 2018; Raychaudhuri and Raychaudhuri 2016; Smith and Colbert 2014). As the first treatment option for SpA, non-steroid anti-inflammatory drugs (NSAIDs) are the mainstay for the management of manifestations of AS and axial SpA (Haroon et al. 2012). Some patients with active disease may need to use biologic disease modifying anti-rheumatic drugs (bDMARDs), which can be assessed by using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) scoring system (Barkham et al. 2005; Garrett et al. 1994). Moreover, patients with active or progressive conditions treated with the drugs mentioned above (NSAIDs and bDMARDs) may be suitable candidates for immunotherapies using antibodies, as discussed below.

5.3.1 Anti-TNF Therapy

Anti-TNF strategies have been based on clinical observations in which TNF levels in the serum of patients with AS were higher than those of healthy subjects for the past several decades (Wendling 2013; Wendling and Toussirot 2004). Currently, there are five antibodies targeting TNF that are available as a therapeutic option for AS: adalimumab, certolizumab pegol, etanercept, golimumab, and infliximab. All of the anti-TNFs presented beneficial effects in phase III randomized double-blind placebo-controlled clinical trials (Dubash et al. 2018).

5.3.2 Anti-IL-17 Therapy

Recent studies of the pathological mechanism of SpA (and also other autoimmune diseases) mediated by the IL-23/IL-17 axis led to the hypothesis that anti-IL-17A therapy shows therapeutic potentiation. Several recent lines of evidence suggest that IL-17A has multiple pathogenic roles in multiple sites, including synovial joints and bones from bone erosion to reformation (Raychaudhuri and Raychaudhuri 2016). In addition to in vitro studies, a clinical trial, the MEASURE study, which assessed an anti-17A antibody, secukinumab, for AS patients, identified a new therapeutic pathway (Baeten et al. 2015; Braun et al. 2017). Since then, the therapeutic feasibility of the IL-17A antagonist ixekizumab has been investigated in phase III clinical trials for skin psoriasis and SpA (Gordon et al. 2016; Mease et al. 2017; Nash et al. 2017).

5.3.3 Anti-IL-23 Therapy

Ustekinumab is a monoclonal antibody targeting p40, a common subunit between IL-12 and IL-23 (Gaffen et al. 2014). As mentioned above, IL-23-IL-23R signaling in specific T cells residing in entheses is sufficient to trigger the SpA-like phenotype in a collagen-antibody-induced arthritis model in mice (Sherlock et al. 2012), with similar conditions to those in patients with AS. To date, ustekinumab has been therapeutically evaluated for skin psoriasis and PsA, as well as Crohn's disease (Feagan et al. 2016). This drug might be beneficial for AS patients, although further studies are needed.

5.3.4 Therapies Targeting Other Molecules

In addition to TNF, IL-17, and IL-23, other proinflammatory molecules have been tested for treatment of SpA in preclinical and clinical studies (Dubash et al. 2018). Tocilizumab, a humanized monoclonal antibody targeting the IL-6 receptor, has been investigated in randomized, placebo-control trials (BUILDER-1 and BUILDER-2) for patients with AS naïve to anti-TNF treatment. However, this antibody failed to result in a significant improvement (Sieper et al. 2014). Similarly, sarilumab, a human monoclonal IL-6 receptor, did not achieve the desired result in a phase II trial (ALIGN study) (Sieper et al. 2015). Rituximab, a monoclonal antibody targeting CD-20, has been used as an anticancer drug and was tested and had a beneficial effect on anti-TNF-naïve-AS patients in a phase II clinical trial (Song et al. 2010).

5.4 Conclusions and Perspectives

SpA is characterized by chronic inflammatory conditions in the joints and spine and shows involvement of the dysregulated IL-23/IL-17 axis with related proinflammatory cytokines mediated by predisposing genetic risk factors, such as HLA-B27, as well as additional environmental conditions (e.g., gut microbial dysbiosis) (Smith and Colbert 2014). Because the beneficial effect of IL-17A inhibition with secukinumab on AS patients was evaluated (Baeten et al. 2013), several antibodies targeting the IL-23/IL-17 pathway have been assessed in clinical trials and resulted in favorable endpoints (Raychaudhuri and Raychaudhuri 2016) (Fig. 5.2). However, there may be some safety concerns for long-term applications of biological therapies, as observed in anti-TNF therapy (Braun and Sieper 2004). Additionally, biological drugs such as passive immunotherapy are costly and burdensome for patients. To address this issue, active immunotherapy can be an option to produce a neutralizing antibody against targeted molecules. The feasibility of therapeutic vaccinations targeting proinflammatory cytokines (e.g., IL-1b, IL-6) for treatment of arthritis and systemic lupus erythematosus (SLE) has been assessed in preclinical and clinical studies (Assier et al. 2017). To date, active immunotherapy alleviating SpA has not yet been reported but may provide patients an additional therapeutic option to enhance their quality of life.

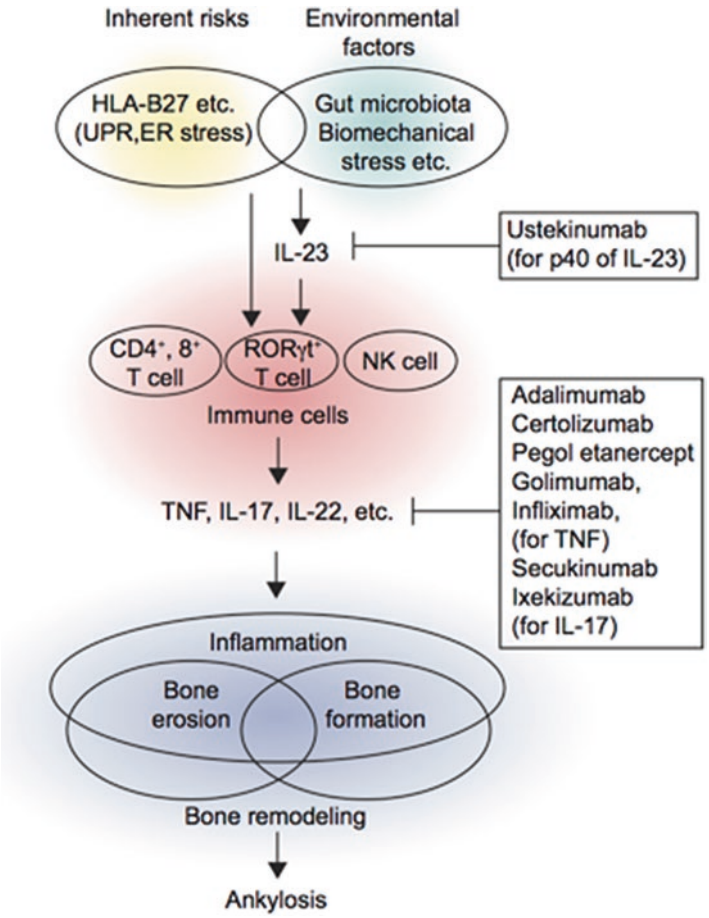

Fig. 5.2 Schematic diagram of the spondyloarthritogenic pathway mediated by the IL-23/IL-17 axis and related immunotherapies
Predisposing risk factors such as HLA-B27 and environmental conditions, including gut dysbiosis and mechanical stress, affect the dysregulation of proinflammatory mediators, such as TNF and IL-17, in part through IL-23 overproduction mediated by RORγt$^+$ T cells residing in entheses. These changes promote local inflammation and bone remodeling and lead to ankylosed spine, which is one of the major hallmarks of ankylosing spondylitis. Immunotherapies targeting the IL-23/IL-17 axis have been developed and are currently being developed. Abbreviations: *HLA* human leukocyte antigen, *ER* endoplasmic reticulum, *UPR* unfolded protein responses, *ROR* retinoid-related orphan receptor, *NK* natural killer, *TNF* tumor necrosis factor

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Chapter 6
Novel Vaccination Tools and Methods

Kunihiko Yamashita

Abstract Vaccination is a traditional method to prevent and cure several infectious diseases or exposure to toxins. At the World Health Organization (WHO) meeting of 2008, Kaddar said that global market projected to rise to USD 100 B by 2025 and more than 120 products are developing (Kaddar M. Global vaccine market features and trends. Epi Seminar April 2008. https://www.who.int/influenza_vaccines_plan/resources/session_10_kaddar.pdf?ua=1. Accessed 12 Jan 2019, 2008).

Vaccine delivery method is strictly defined for each vaccine. In general, vaccines are administrated by intradermal, subcutaneous, and intramuscular mode of delivery by a traditional needle syringe, except for Polio vaccine, which is administrated by oral or needle syringe injection. Recently, several new vaccine-delivering devices, administration roots, and methodology have been proposed and are being developed, such as the microneedle device, needle-free injector device, intranasal spray root, and DNA vaccine (National Institute of Health. Researchers develop microneedle patch for flu vaccination. https://www.nih.gov/news-events/news-releases/researchers-develop-microneedle-patch-flu-vaccination. Accessed 12 Jan 2019, 2017; U.S. Food & Drug Administration. FDA updated communication on use of jet injectors with inactivated influenza vaccines. https://www.fda.gov/BiologicsBloodVaccines/Vaccines/QuestionsaboutVaccines/ucm276773.htm. Accessed 12 Jan 2019, 2014; U.S. Food & Drug Administration. FluMist Quadrivalent. https://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm293952.htm. Accessed 12 Jan 2019, 2018). Here, we describe the new methods, especially emphasizing on recent reports of their operating principle and clinical study application.

Keywords Microneedle · Needle-free injector · Electroporation · Vaccine

K. Yamashita (✉)
Department of Impulse Science for Medicine, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan
e-mail: ku_yamashita@impluse.med.osaka-u.ac.jp

H. Nakagami (ed.), *Therapeutic Vaccines as Novel Immunotherapy*,
https://doi.org/10.1007/978-981-32-9628-2_6

6.1 Microneedle Delivery System

Microneedle devices focus on delivering the medicine into the epidermis and/or dermis. The skin epidermis and dermis are attractive sites for vaccination because a lot of antigen-presenting cells (APCs), such as epidermis Langerhans (LH) cell and dermis dendritic cell (DC), are located in the skin. For example, LH cells account for about 1–2% of epidermal cells (Seneschal et al. 2012; Abd Warif et al. 2015).

Microneedle devices are of four types: (i) solid microneedles that can increase skin permeability of the medicine, (ii) hollow microneedles for drug infusion into the skin, (iii) drug-coated microneedles that dissolves drugs into the skin, and (iv) a drug-encapsulated biodegradable polymer microneedles that fully dissolve in the skin (Fig. 6.1) (Kim et al. 2012).

Several systems and materials were proposed and are still developing. Chen et al. reported a pump-driven microneedle drug-delivering model to improve the hollow microneedle, and Kim et al. reported application of biodegradable microneedle for perivascular drug delivery (Chen et al. 2016; Kim et al. 2017). Lin et al. reported hollow microneedle-mediated intradermal delivery of polymeric nanoparticles (NPs). This vaccine system had a different pharmacokinetic profile compared to intravenous or subcutaneous administration, and this combination was said to be a promising approach to improve vaccination (Niu et al. 2019). Gala et al. investigated the vaccination potential of the formalin-fixed whole-cell *Neisseria gonorrhoeae* encapsulated in biodegradable microneedle and showed the possibility of it as a vaccine to prevent gonorrhea infection (Gala et al. 2018).

In addition, safety, tolerability, acceptability, and immunogenicity of microneedle patch influenza (flu) vaccine has also been reported (Fernando et al. 2018). Chen et al. reported that Bacillus Calmette–Guérin (BGC) vaccination to the epidermis via microneedle elicits a comparable immune response, but does not cause a harmful

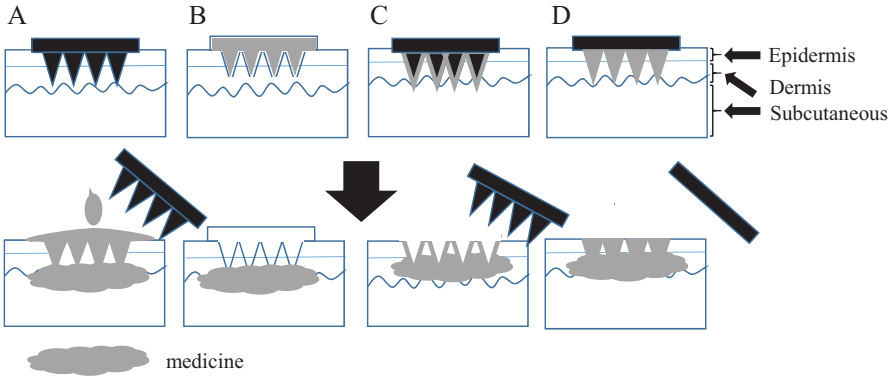

Fig. 6.1 (**a**) The microneedle makes minute punctures and the medicine is applied through the surface of the skin. (**b**) The medicine is released through the hollow device. (**c**) The medicine-coated microneedle set to the skin surface and medicine dissolves into the skin. (**d**) The medicine is included in the biodegradable needle, which dissolves into the skin

skin reaction compared to traditional intradermal vaccination (Chen et al. 2017). Furthermore, several clinical studies for assessing the efficacy of microneedle injection are listed in the database of clinicaltrials.gov (U.S. National Library of Medicine; www.clinicaltrials.gov accessed on 17 January 2019), such as No. NCT01737710 and NCT01368796. In the NCT01737710 clinical study, the flu vaccine was administrated to non-atopic participants and those with atopic dermatitis. In the NCT01368796 study, the flu vaccine was administrated by several methods to investigate the protectiveness via different testing methods (including the microneedle) and for acceptability. These trials indicated that the microneedle device has the potential to replace some of the vaccination methods in the near future.

6.2 Needle-Free Injector

Needle-free injector is a new drug delivery system for transdermal administration performed by high force via spring, charged gas, or Lorentz force (Ravi et al. 2015). The mechanism of injection is quite simple, generating high-speed liquid flow (usually >100 m/sec) to make a pin hole (0.1–0.2 mm) on the skin surface, and diffusing the liquid into the body. The depth of diffusion area is defined by the force of each device. Interestingly, when the needle-free injector is applied with powder, the administrated area is larger than that applied for liquids (Al-kaf and Othman 2017). This system is expected to have several merits compared to the traditional hypodermic syringe; it may prevent infection from needle-stick injuries in medical staff, and it allows a less painful administration than the traditional needle syringe. In addition, different pharmacokinetic and pharmacodynamic variabilities are observed when applied as insulin injection compared to traditional needle syringe. Needle-free injectors for administering insulin are currently in practical use (Engwerda et al. 2017; Guo et al. 2017; The needle free alternative 2019; Minnesota Rubber and Plastics 2019).

The needle-free devices can be classified in two or three classes based on their mechanism of the power source for drug delivery. In recent years, several needle-free systems are available, such as Biojector® 2000, ZetaJet™, Pharmajet Stratis®, Penjet®, Medi-Jector VISION®, among others. Thus, it is possible to administrate highly viscous drug products and powder which is difficult or impossible to administer by traditional needle. The typical needle-free injectors are summarized in Table 6.1. Needle-free injectors can deliver medicine to skin or muscle region, but it is impossible to direct intravenous injection with this system. In addition, the direction of force from skin surface to muscle is straight; thus, the technical difficulty of accurate intradermal injection still remains, especially in small experimental animals. In fact, only three of nine injectors shown in Table 6.1 are capable for intradermal injection, whereas all injectors are capable for subcutaneous and intramuscular injections. However, needle-free jet devices are promising new tools, for which the clinical use and several investigations for vaccination use are ongoing.

Table 6.1 Needle-free injectors currently available

Product name	Company	Power source	Target region	Volume (ml)	Application or comments[a]
PharmaJet Stratis®	PharmaJet	Spring	SC/IM	0.5	Vaccine/DNA vaccine Flu
Medi-Jector VISION®	Antares Pharma	Spring	SC	–	Insulin
ZetaJet™	Inovio Pharmaceuticals, Inc.	Spring	SC/IM	0.05–0.5	Vaccine/DNA vaccine
Biojector® 2000	Inovio Pharmaceuticals, Inc.	Compressed gas (CO2)	ID/SC/IM	~1	For ID administration, spacer is needed
PenJet®	PenJet Corporation	Compressed gas (N2)	SC/ID/IM	0.1 or 0.5	Lyophilized drug
J-Tip™	National Medical Products, Inc.	Compressed gas (CO2)	SC	0.25	Lidocaine
ZENEO®	Crossject	Pyro-generated gas	SC/IM	–	Midazolam Sumatriptan
BENTObox Injection System	Portal Instruments	Lorenz force	SC	−1	
Actranza™ lab	Daicel Corporation	Pyro-generated gas	ID	0.01–0.1	For mouse and rat

[a]Application or comments information are cited from company's home page
ID intradermal, *IM* intramuscular, *SC* subcutaneous

6.2.1 Vaccination via Needle-Free Injector

Several investigations on vaccination using needle-free injectors have been reported. Ambuel et al. applied the needle-free injector to dengue virus vaccination, via which the live-attenuated tetravalent dengue vaccine induced neutralizing antibody responses and successfully prevented the viral infection in non-human primates (Ambuel et al. 2014). Furthermore, Nikolas et al. reported that a glycoconjugate meningococcal group Y vaccine, delivered by needle-free injector, induced functional protective antibody responses in vivo of similar magnitude to the conventional needle vaccination which contained an alum adjuvant (Weissmueller et al. 2017).

In the practical vaccination use, the PharmaJet Stratis® was approved by Food and Drug Administration (FDA) and utilized for seasonal flu vaccination (FDA Updated Communication 2019). Other clinical studies which applied needle-free injectors for dengue virus vaccine, BCG vaccination, human papilloma virus vaccine, and poliomyelitis are listed in the database of the clinicaltrials.gov (U.S. National Library of Medicine; www.clinicaltrials.gov accessed on 17 January 2019) as NCT01728792, NCT01742364, NCT01924754, and NCT01847872, respectively. In addition to these studies, several clinical studies are also listed in the database.

6.3 DNA Vaccination Devices and Methods

DNA vaccination is promising as new vaccine strategy, as it appears to have several merits compared to the traditional vaccines, such as attenuated virus vaccine or pathogen- or toxoid-based vaccines. To prepare the DNA vaccine, there is no need to cultivate the pathogen for antigen preparation, and a DNA plasmid, which is suitable for industrial production under Good Manufacturing Practice (GMP) conditions, is required (World Health Organization WHO Technical Report Series No 941 2007). In addition, reconstruction of antigen expressing plasmid and co-expression with other genes, such as immune-stimulating genes, are relatively easy. Therefore, DNA vaccine is capable of responding to antigen mutation and has the potential to enhance vaccination ability.

However, the DNA plasmid should be introduced into the cell and nucleus to express the coded antigen for effective vaccination. In this aspect, traditional needle had a very low potential. When needle-free injector is used for DNA plasmid injection, the gene expression of luciferase is about 10–100 times higher than the traditional needle syringe (Cartier et al. 2000; Babiuk et al. 2003). Furthermore, the antibody production level is higher when needle-free injector is employed compared to traditional needle syringe, suggesting that antigen production level is related the gene expression level (Kwilas et al. 2014; Graham et al. 2013). In addition, the antigen-coded gene should be expressed into or near the APCs; thus, the ideal DNA vaccination device should have the ability to not only inject DNA plasmid into the epidermis and dermis but also to introduce it into the cell and nucleus.

In terms of the DNA introduction into the cell and nucleus, electroporation method is well established and in vivo application for DNA vaccination is developing. For this purpose, new medical devices, such as CELLECTRA® (Inovio Biomedical Corporation) and TriGrid™ Delivery System (ICHOR Medical Systems Inc.), are being developed (Amante et al. 2015; Ichor Medical Systems 2019). The combination of a traditional needle syringe with electroporation has commonly been employed in in vivo electroporation, and the efficacy for DNA vaccination has been reported (Cashmana et al. 2017; Elizaga et al. 2018). In addition, Biliana et al. reported that intradermal electroporation has the adjuvant effect by inducing the production of pro-inflammatory cytokines and significantly increasing local concentrations of transforming growth factor (TGF)-alpha and interleukin (IL)-12 (Todorova et al. 2017). However, the effectiveness of needle-free injector combined with electroporation for vaccination is not fully validated. David et al. reported the combination could improve the dose restriction of DNA vaccine (Hallengärd et al. 2012). However, Shawn reported that when the luciferase expression plasmid was injected into porcine skin, the order of gene expression based on the mode of delivery was as follows: needle-free injection + electroporation $\geqq$ needle free injection > traditional needle. Nilsson et al. reported that when the human immunodeficiency virus (HIV) prime-boost DNA-MVA-rgp140/GLA vaccines were tested in a human clinical study, a significant difference was observed between HIV-DNA Intradermal

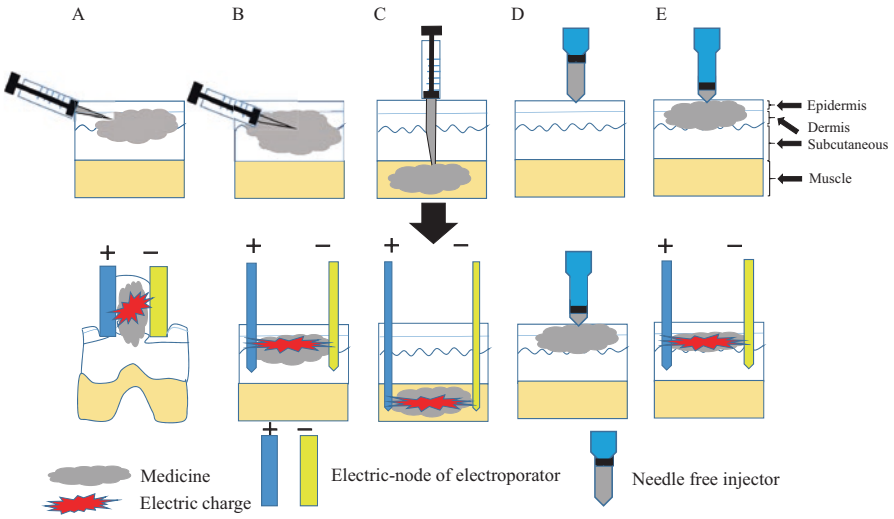

Fig. 6.2 (**a**) The medicine is injected into the intradermal and/or subcutaneous region. Next, the injected region is clipped using electro-node and electric charged. (**b**) The medicine is injected into the intradermal and/or subcutaneous region. Next, the injected region is pierced by electro-node and electric charged. (**c**) The medicine is injected into the muscle region. Next, the injected region is pierced by electro-node and electric charged. (**d**) The medicine is filled into the needle-free injector and injected into the intradermal and/or subcutaneous region or muscle region by a power generator (in blue). (**e**) The medicine is filled into the needle-free injector and injected into the intradermal and/or subcutaneous region or muscle region. Next, the injected region is pierced by electro-node and electric charged. (**d** and **e** indicate the case of intradermal injection)

injection(ID)/Electroporation recipients and HIV-DNA ID recipients in immune response using ELISpot assay (Babiuk et al. 2003; Viegas et al. 2018). The typical methods for DNA vaccination are shown in Fig. 6.2.

DNA vaccination devices and methods are being investigated in several aspects. For the purpose of anti-pathogen infection, the animal experiments of DNA vaccination for influenza virus, hepatitis C virus, HIV, rabies virus, and malarial parasites have been reported (Andersen et al. 2017; Lee et al. 2017; Hu et al. 2017; Ullas et al. 2014; Datta et al. 2017). Recently, the clinical study of DNA vaccination for Zika virus and Hepatitis B virus were reported and are listed in the database of U.S. National Library of Medicine as NCT01728792 and NCT03463369, respectively (Yang et al. 2017; Gaudinski et al. 2017; Abbink et al. 2018). In addition to preventing pathogen infection, anti-tumor DNA vaccination therapy is also being investigated. A total of 136 clinical trials for cancer DNA vaccine are currently listed in the database of ClinicalTrials.gov (www.clinicaltrials.gov accessed on 17 January 2019). Interestingly, the microneedle patch device and traditional needle syringe, as well as the needle-free injector and electroporation, are being used in combination with several plasmid designs and other supporting materials, such as nanoparticles, liposomes, or positively charged polymers for DNA vaccination.

Liao et al. reported that the microneedle formed by combination of DNA vaccine with positively charged polymers could induce antibodies in mice experiments (Liao et al. 2017).

These indicate that new DNA vaccination tools and methods are promising for the development of new therapeutic strategies.

6.4 Conclusion

Nowadays, vaccination medicines are mainly administrated by needle syringe. However, new alternative vaccination methods are developing with new devices, such as microneedle patches, needle-free injector, intranasal sprays, and others. In addition to device developments, several new vaccination tools, such as molecular adjuvants, are also being investigated. Thus, it is essential to comprehend the potential of each vaccination device and utilize its combination with medicines to advance the field of vaccinology.

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Chapter 7
Translational Research of Novel Peptide Vaccine

Hideki Tomioka, Akiko Tenma, and Makoto Sakaguchi

Abstract Peptide vaccines are in development as potential therapies for many major conditions, including chronic viral infections, allergies, cancer, Alzheimer's disease, diabetes, hypertension, obesity, and rheumatoid arthritis. The therapeutic vaccines are known to induce neutralizing antibodies against self-molecules and have been clinically tested.

Active immunotherapy has been the most extensively studied approach in Aβ-targeted therapy. To improve the immunogenicity and reduce the adverse event, in the past decade progress of active immunotherapy has been made both in the selection of B cell epitope and the carrier protein. Progress made in peptide-based vaccinations to induce antibody has invigorated the search for vaccine modalities.

Keywords Peptide vaccine · Active immunotherapy · Carrier protein · B cell epitope

7.1 Vaccine

Edward Jenner developed the preventive medicine by taking advantage of the similarities between cowpox and small pox viruses. He used the cowpox virus to confer protection against smallpox, a related virus, in human. In the nineteenth and twentieth centuries, the base of immunotherapy was developed. In this era, the vaccines to prevent rabies and plague were developed. Vaccines are biological preparation that improves immunity to a particular disease. Vaccines are administered to healthy individuals to prevent infection. By introducing a part of the virus or an inactive

H. Tomioka (✉) · A. Tenma · M. Sakaguchi
FunPep Co., Ltd, Ibaraki, Osaka, Japan
e-mail: htomioka@funpep.co.jp

H. Nakagami (ed.), *Therapeutic Vaccines as Novel Immunotherapy*,
https://doi.org/10.1007/978-981-32-9628-2_7

virus into body, the immune system reacts by producing antibodies. Vaccines works by making us produce antibodies to fight disease without actually infecting us with disease. The induction of antibodies by vaccination against infection diseases has been the most effective medical intervention in human history.

Over the last three decades, monoclonal antibodies have made a dramatic transformation from scientific tools to powerful human therapeutics. At present, more than 70 therapeutic monoclonal antibodies are marketed in the world. A vaccine may also confer passive immunity by providing antibodies or lymphocytes made by an animal or human donor.

7.2 Active Immunotherapy

Today, therapeutic vaccines are in development for many major conditions, including chronic viral infections, allergies, cancer, Alzheimer's disease, diabetes, hypertension, obesity, and rheumatoid arthritis. Therapeutic vaccines can be categorized into two groups, those that induce antibodies (active immunotherapy) and those that induce T cells, mostly cytotoxic T lymphocytes (CTLs). The therapeutic vaccines is known to induce neutralizing antibodies against self-molecules. Table 7.1 summarizes active immunotherapies based on this strategy that have been clinically tested. The first vaccine clinical trials for AD patients were started with an Aβ peptide vaccine (AN1792) (Gilman et al. 2005). In this clinical study, 6% of the patients developed meningoencephalitis. It was suggested that vaccination with Aβ peptide in a Th1-type adjuvant (QS-21) may induce T cell response against Aβ, which would result in the development of meningoencephalitis. The strategy to avoid T cell response against Aβ is developed such as adjuvants, immunogens, carrier protein, and different routes following clinical trials. T cells respond to peptide epitopes presented on major histocompatibility complex (MHC) class I or class II molecules. The minimal length of a peptide that can be bind to MHC class I, otherwise phrased, recognized by cytotoxic T lymphocytes (CTLs) is 8 amino acids, and MHC class II molecules that is recognized by Th cells is 10–12 amino acids. Peptides <8 amino acids are unable to induce T cell responses.

Induction of antigen-specific humoral immunity is a primary vaccine goal. The search of B cell epitopes to induce antibodies is important. The developments in bioinformatics, proteomics, immunogenomics, structural biology, and other sciences have spurred the growth of vaccinomics where computer-assisted approaches serve to identify suitable peptide targets for eventual development of vaccines (Nandy and Basak 2016).

This principal has underpinned the design of second-generation therapeutic AD vaccines, which utilize small Aβ peptide fragment conjugated to either VLP (CAD106) (Wiessner et al. 2011; Winblad et al. 2012). The clinical study of Lu AF20513 for Alzheimer's disease was done (Davtyan et al. 2013). In this transla-

Table 7.1 B cell vaccines targeting self-molecule

Target	Indication	Antigen	Organization
Ab1–40/42	Alzheimer's disease	$A\beta_{1-42}$	Wyeth/Elan
		$A\beta_{1-7}$–CRM197	Pfizer/Janssen
		$A\beta_{1-6}$–Qb	Novartis/Cytos
		$A\beta_{Nterm}$ (n.d.)/Isocomatrix	Merck Co.
		Affitope $A\beta_{1-6}$	Affiris/GSK
		$A\beta_{1-14}$-UBITh peptide	United Biomedical
		$A\beta_{1-15}$/Liposome	AC Immune
Angiotensin I/II	Hypertension	Angiotensin II-Qb	Cytos
		Angiotensin I-KLH	Protherics
CETP	Hyperlipidemia	$CETP^{aa461-476}$ fused to $TT^{aa830-843}$ peptide	Avant
hCG	Contraception	b-HCG – TT	Indian Government
FSH		FSH	Indian Government
	Prostate cancer	GnRH-DT	Aphton
EGF	NSC-lung cancer	hEGF – TT (inactivated)	Micromet/Cancer Vax
Her2	Breast cancer	Truncated HER2 fused to TT epitopes	Pharmexa
Gastrin	Pancreatic cancer	Gastrin 17^{aa1-9} – DT	Aphton
Mucin	Cancer	Sialosyl-Tn-KLH	Oncothyreon/ Merck KGaD
Ghrelin	Obesity	Ghrelin $^{aa1-8}$ – Qb	Cytos
IgE	Allergic asthma	CH2 CH3 CH4 oposum human hybrid constant domain of IgE	Resistentia
IL1β	Type II diabetes	IL1b mutein – Qb	Cytos
IFNα	Systemic lupus erythematosus	rINFα–KLH (inactivated)	Neovacs
TNFα	Rheumatoid arthritis	rTNFα–KLH (inactivated)	Neovacs
	Crohn's disease	rTNFα–KLH (inactivated)	Neovacs
	Cachexia	rTNFα with internal TT T-cell epitopes	Pharmexa[74]
	Psoriasis	TNF pep.–Qb	Cytos

TT tetanus toxoid, *DT* diphtheria toxoid, *Qb* Qbeta VLPs, *KLH* keyhole lympet hemocyanin-means chemically conjugated

tional study, they have devised and validated a novel AD epitope vaccine, Lu AF20513, in which the T-helper (Th) cell epitopes of Aβ42 were replaced by two foreign Th epitopes from tetanus toxoid (TT), P2, and P30, and the immunodominant B cell epitope of amyloid Aβ1–12. And also this type of vaccine minimizes possible cross-reactivity because the peptide sequences used are short and are target specific.

7.3 Carrier Protein

Carrier proteins are also important to achieve the induction of antibody. Carrier proteins are categorized into three groups: (1) Toxoid protein derived; CRM197: diphtheria toxin cross-reactive mutant, Qb: bacteriophage Qbeta VLPs, OMPC: *N. meningitidis* outer membrane protein complex, TT: tetanus toxoid, DT: diphtheria toxoid. (2) Potently immunogenic protein; KLH: keyhole limpet hemocyanin. (3) Helper T cell peptide epitopes; UBITh: helper T cell technology, $TT^{aa830–843}$ peptide, TT T-cell epitope. The carrier protein introduces a potential for undesirable immune responses such as allergic and autoimmune reactions. The large peptide-carrier protein elicits irrelevant immune responses predominantly misdirected to the carrier protein rather than the target site (Cease et al. 1987). B cell epitopes linked to different helper T cell peptide epitopes as a chimeric peptide is unique strategy. United Biomedical, Inc. has developed a set of core technologies for the discovery of synthetic peptide-based immunotherapeutic and vaccines (Wang and Walfield 2005). Carrier proteins such as KLH and linkage are not required.

General vaccines against pathogens (i.e., bacteria, virus) strongly induce the activation of innate immunity, which leads to the cell surface antigen interaction between antigen-presenting cells (APCs) and T cells. The APCs phagocytose the vaccines and present a T-cell epitope to T cells through the major histocompatibility complex (MHC) classes I and II. Coactivation of innate immunity and antigen presentation induce T cell activation, resulting in both cytotoxic T cell via MHC class I and antibody production via MHC class II. The cytotoxic T cell activation is very important for the immune elimination such as infections and cancer; however, the vaccine against self-antigen is required to induce not cellular immunity but humoral immunity from the point of view of safety concerns. As MHC classes I epitopes usually consist of 8–10 amino acids, short peptides (<8 amino acids) are preferred as antigens to avoid the induction of the cellular immunity via MHC class I. On the other hand, the reaction against self-antigen is tightly controlled via the repression of self-reactive T cells in the human immune tolerance system. However, self-reactive B cells are still active and can be induced by T-cell activation. Thus, efficient antibody production by B cells requires helper T cell activation, to prevent immune tolerance. To fully activate B cells, CD4+ T cells must firstly differentiate into plasma and memory cells. Because of T cell immune tolerance, self-reactive B cells, albeit responsive to antigens, cannot function without the help of CD4+ T cells, targeting the self-antigen. As short peptide antigens do not include the T cell epitope, T cells cannot be activated, and B cell-induced antibody production does not occur. To overcome this problem, peptide antigens may be used in combination with foreign T-cell epitopes, which results in antibody production (Nakagami and Morishita 2018).

Antibody-inducing peptide that is named by FunPep Co., Ltd. contains two amino acid sequences which are classified into helper T cell epitope (AJP001 peptide) or B cell epitope. ε-aminocaproic acid (Ahx) is inserted as spacer between these epitopes and N- or C-terminal amino acid is acetylated and amidated, respec-

tively. Antibody-inducing peptide is taken up by antigen-presenting cells such as dendritic cells or B cells and is presented on the cell surface in association with MHC class II molecule. Naïve CD4+ T cells that have TCR that binds to the AJP001-MHC class II complex are activated with co-stimulatory molecule because AJP001 itself can activate the innate immune system through the activation of inflammasome and NF-κB pathway. Activated Th cells recognize B cells presenting AJP001 bound to MHC class II; subsequently, B cells are activated and induced to produce antibody.

The monoclonal antibodies (mAbs) approved in the last 20 years by the FDA across disease states. The annual price of mAb therapies is about $100,000 higher in oncology and hematology than in other disease states (Hernandez et al. 2018). More than 13 clinical studies were done to target the self-molecules. The vaccine platform based on the design of minimal subunits, using synthetic peptides, has the potential to deliver precisely defined epitopes that can be produced at large-scale, high yield, and relatively low cost. Antibody-inducing peptides are expected as alternative excellence of technology to supply inexpensive drugs for current expensive antibody drugs. The PI/IIa clinical trial of FPP003 that is AJP001-B cell epitope complex compound has been started at Australia in 2019 (https://www.funpep.co.jp/en/archives/180). Progress made in peptide-based vaccinations to induce antibody has invigorated the search for vaccine modalities.

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Chapter 8
Closing: Clinical Applications of Therapeutic Vaccines in the Near Future

Hironori Nakagami and Ryuichi Morishita

Abstract The new technology available for vaccine development allows clinicians to administer vaccines to treat chronic diseases, such as dementia and high blood pressure. A vaccine for Alzheimer's disease targeting the amyloid β and tau proteins and a high blood pressure vaccine targeting the renin-angiotensin system have been developed and advanced to clinical trials. When these vaccines are established in the future as therapeutic options for chronic diseases, their administration several times a year will replace a daily medication. Since social security expenses have increased along with social problems in our country, which has seen rapid increases in the aging population, these vaccines may provide cost benefits. Furthermore, improvements in medicine adherence might be achieved as a clinical merit of the vaccine treatment for chronic diseases. Because the number of aging patients using multiple drugs (polypharmacy) has increased, the management of administered drugs requires extensive human resources. If a vaccine for chronic diseases can decrease the number of drugs taken by patients, it will contribute to improvements in social problems. Moreover, an anti-inflammatory cytokine vaccine has been developed for chronic inflammatory diseases, which will be able to replace antibody therapy in the future.

Keywords Vaccine · Innate immunity · Adjuvants · T cells · Antibody

H. Nakagami (✉)
Department of Health Development and Medicine, Osaka University, Graduate School of Medicine, Suita, Japan
e-mail: nakagami@gts.med.osaka-u.ac.jp

R. Morishita
Department of Clinical Gene Therapy, Osaka University Graduate School of Medicine, Suita, Japan

H. Nakagami (ed.), *Therapeutic Vaccines as Novel Immunotherapy*,
https://doi.org/10.1007/978-981-32-9628-2_8

8.1 History of the Development of a High Blood Pressure Vaccine

The development of vaccines for high blood pressure has a long history, and the renin-angiotensin system has been extensively studied as a marker (Helmer et al. 1958; Wakerlin 1958). The first trial reported in the 1980s was a vaccine for renin, which is the upstream target of the renin-angiotensin system. In this experiment, the renin vaccine consisted of a dog or human renin protein and was administered to mice. As a result, the blood pressure was significantly decreased (approximately 30 mmHg), but damage to the kidney tissue with inflammation was observed (Michel et al. 1987, 1990). These findings highlight the unsolved problem of safety issues. In the 1990s, a vaccine against angiotensin I or II was developed. Since angiotensin I or II is a short peptide that only comprises 10 or 8 amino acids, respectively, the vaccine consisted of the peptide conjugated to a carrier protein to strengthen the immune response. Angiotensin I was fused to part of the clostridium tetanus toxoid (TT) protein (PMD-2850) to create a vaccine and administered to rats with alum adjuvants (Gardiner et al. 2000). The vaccination of PMD-2850 with alum on days 0, 21, and 42 (three times) resulted in a significant increase in the antibody titer and a significant inhibition of angiotensin I-induced increase in blood pressure, without any toxic effects. Another type of vaccine was created in which angiotensin I was conjugated to keyhole limpet hemocyanin (KLH) as a carrier protein (PMD-3117). The effects of these two vaccines for angiotensin I on rats were compared (Downham et al. 2003). As a result, both vaccines induced a similar immune response and inhibited the angiotensin I-induced increase in blood pressure. Based on these experiments, the first clinical trial of an angiotensin I vaccine (PMD-2850 and 3117) was designed in 2003 (Brown et al. 2004). Healthy male volunteers who were treated with a single dose of both angiotensin I vaccines did not display an anti-angiotensin I IgG response in a Phase Ia clinical study. However, an anti-carrier protein IgG response was observed in subjects treated with the highest TT conjugate vaccine dose and each of the Angiotensin I–KLH conjugate vaccine doses investigated. The two highest dose groups showed a maximum IgG response to KLH 21 days after the administration of the single conjugate vaccine dose. Furthermore, two-dose immunization with angiotensin I–KLH conjugate vaccine (Phase Ib clinical study) of healthy male volunteers induced the production of anti-angiotensin I IgG. At 21 days after the second angiotensin I–KLH conjugate vaccination (50 μg), the anti-angiotensin I IgG titers were markedly increased. No statistically significant effect of treatment was observed on blood pressure. The anti-angiotensin I IgG titers observed in human clinical trials were lower than the titers observed in the rat studies. This finding may explain the lack of effect of the immunization on blood pressure responses.

8.2 Clinical Trials of Angiotensin II Vaccines

Using a similar logic to angiotensin I, an angiotensin II vaccine was conjugated to a virus-like particle (VLP) as a carrier protein (CYT006-AngQb). The administration of an angiotensin II vaccine (CYT006-AngQb) significantly increased anti-angiotensin II antibody titers in spontaneously hypertensive rats (SHR) (Ambühl et al. 2007). Based on these experiments, the effect of CYT006-AngQb was evaluated in a multicenter, double-blind, randomized, placebo-controlled clinical trial (Phase IIa) (Tissot et al. 2008) Seventy-two patients with mild to moderate hypertension were randomly assigned to receive subcutaneous injections of either 100 μg or 300 μg of the vaccine (CYT006-AngQb) or placebo at weeks 0, 4, and 12. Each group comprised 24 patients. The 24-h ambulatory blood pressure was measured before treatment and at week 14. Blood pressure was unaffected in the low dose group (100 μg of vaccine) and the placebo group, but was significantly decreased in the high dose group (300 μg of vaccine). In the latter patients, the mean ambulatory daytime blood pressure was reduced by −9.0/−4.0 mmHg at week 14 compared to the baseline level. Interestingly, the 300 μg dose reduced the early morning blood pressure surge. During the evaluation of safety issues, five serious adverse events were reported (two in the 100 μg group, two in the 300 μg group, and one in the placebo group); none were deemed to be treatment related. Most of the side effects were mild, namely, transient reactions at the injection site. This study was the first to report a successful reduction in blood pressure using vaccine therapy, with no serious adverse events. However, further development of the study failed to reproduce these results. Notably, an accelerated immunization schedule was employed (0, 2, 4, 6, and 10 weeks) in an attempt to induce higher antibody titers than those observed with the initial protocol (0, 4, and 12 weeks). The authors concluded that the accelerated regimen may have led to the induction of high titers of antibodies with low affinities for the antigen. However, the involvement of this factor in the lack of therapeutic effect is hard to confirm. Another angiotensin vaccine using a novel adjuvant, CoVaccine HT™, was also investigated in a randomized, double-blind, placebo-controlled Phase II clinical trial; however, this study was terminated due to dose-limiting adverse effects (from clinicaltrials.gov).

Our group has developed a therapeutic vaccine for angiotensin II and showed its efficiency in several animal models (Nakagami et al. 2013; Koriyama et al. 2015; Wakayama et al. 2017). A clinical trial (Phase I/IIa) using the angiotensin II DNA vaccine (AGMG0201) has recently begun in Australia. The aim of the study is to evaluate the safety and efficiency of this novel vaccination protocol. In addition to the antibody titer and blood pressure, safety measures and blood and urine tests will be performed, and adverse events (AEs) will be monitored. The study employs a randomized, double-blind, and placebo-controlled design. The stability of the antibody titer will be evaluated over at least 6 months, and the association between antibody titer and blood pressure will be verified to evaluate the efficacy. The trial is currently in progress and may provide us with important insights into the future development of therapeutic vaccines.

8.3 Anti-cytokine Vaccine for Rheumatoid Arthritis

In addition to vaccines for high blood pressure, several therapeutic vaccines have been developed for translational research (Fig. 8.1). For patients with chronic inflammatory diseases, such as rheumatoid arthritis (RA) or Crohn's disease, antibody therapy is very effective at decreasing the symptoms of the disease. An anti-inflammatory cytokine vaccine has recently been developed for chronic inflammatory diseases, which may replace the antibody therapy in the future. Active immunization against TNF (tumor necrosis factor)-α with TNF-Kinoid has been reported as a TNF-α vaccine (Semerano et al. 2011; Biton et al. 2011). This vaccine technically reduces the immune tolerance to human TNF-α (hTNF-α) and leads to the production of neutralizing polyclonal antibodies in patients. TNF-α Kinoid is a heterocomplex vaccine that consists of immunogenic hTNF-α conjugated to a carrier protein, keyhole limpet hemocyanin (KLH) (Harris et al. 1999). The therapeutic effect of TNF-α Kinoid is evaluated in hTNF-α transgenic mice, which overexpress hTNF-α and spontaneously develop arthritis at 6–8 weeks of age (Hayward et al. 2007). As a result, an early anti-hTNF-α immunization protected hTNF-α transgenic mice from developing arthritis. Thus, TNF-Kinoid efficiently blocks the function of hTNF-α during the development of arthritis (Le Buanec et al. 2006). Importantly, TNF-Kinoid does not sensitize T cells to native hTNF, and endogenous TNF does not boost the immune response. In addition, the anti-hTNF antibody titers display a bell-shaped curve over time (Delavallée et al. 2008, 2009). According to these results, clinical trials have been designed for patients with Crohn's disease (EudraCT number 2010-019996-32) and RA (EudraCT number 2009-012041-35) using TNF-Kinoid.

A pilot study was designed for patients with RA who previously experienced a secondary failure to respond to TNF antagonists. Patients were intramuscularly immunized with 2 or 3 doses of the placebo (n = 10) or three different doses of

Target Molecule	Target Diseases	Formulation of vaccine
Amyloid β	Alzheimer's disease	Amyloid $\beta_{aa1-6,\ 1-7,\ 1-14}$ with several carrier proteins
Tau	Alzheimer's disease	Tau-peptide-KLH (AADvac1)
Alpha synuclein	Parkinson disease	α-synuclein peptide (PD01A)
Angiotensin II	Hypertension	AngII-Qb (VLP: virus like particle)
PCSK9	Dyslipidemia	PCSK9 peptide-KLH
hCG	Contraception	HCG – TT (Tetanus Toxide)
GnRH	Prostate cancer	GnRH-DT (Diphtheria Toxide)
EGF	NSC lung cancer	hEGF – TT
HER2	Breast cancer	Truncated HER2 fused to TT epitopes
Gastrin	Pancreatic Cancer	Gastrin $_{aa1-9}$ – DT
Ghrelin	Obesity	Ghrelin $_{aa1-8}$ – Qb
IgE	Allergic asthma	CH2-CH3-CH4 of IgE
IFN-α	HIV, AIDS, SLE	rINF-α–KLH (inactive)
TNF-α	Rheumatoid arthritis	TNF-kinoid
	Crohn's disease	TNF-kinoid

Fig. 8.1 Preclinical and clinical trials of B-cell type vaccine
Target molecule, target diseases, and formulation of vaccine were shown

TNF-Kinoid (90 μg; n = 6, 180 μg; n = 12, or 360 μg; n = 12). The highest anti-TNF antibody response was detected in patients immunized with 360 μg of TNF-Kinoid in three injections, although this difference was not significant compared with all other groups. The TNF-K therapeutic vaccination induced the production of anti-TNF antibodies in patients with RA in a dose- and schedule-dependent manner and was well tolerated. Interestingly, patients who developed anti-TNF antibodies showed a trend toward clinical improvements induced by TNF-Kinoid (Durez et al. 2014). Further modifications will be necessary to verify the clinical benefits of TNF-Kinoid before its clinical application.

8.4 Applications of Tailored Medicines in the Future

The ultimate goal of a therapeutic vaccine is a tailored medicine to prevent the onset of diseases in the future. In the research field of high blood pressure, we will introduce the following preventive medicines to spontaneously hypertensive rats (SHRs). Angiotensin-converting enzyme inhibitors (ACEis) or angiotensin receptor blockers (ARBs) are administered for a short time to a young SHR before an increase in blood pressure is detected, and the blood pressure of SHRs was monitored after termination of the medication to examine a persistent effect of the ACEi/ARB. During treatment with ACEi/ARB, blood pressure was significantly decreased compared with non-treated SHRs. Interestingly, after the medication was terminated, blood pressure was still significantly decreased compared with non-treated SHRs (Nakaya et al. 2001). Based on these results, the blockade of the renin-angiotensin system will delay the development of high blood pressure by restraining some reactions before the blood pressure increases in patients. Similarly, the clinical study TROPHY was designed to examine whether an ARB treatment for prehypertension prevents or postpones stage 1 hypertension (Julius et al. 2006). In this study, prehypertension was defined as a systolic pressure of 130–139 mmHg and diastolic pressure of <89 mmHg, or a systolic pressure of <139 mmHg and a diastolic pressure of 85–89 mmHg. Patients were randomly assigned to receive 2 years of ARB or placebo treatment, followed by 2 years of placebo. Seven hundred and seventy-two participants (391 in the candesartan group and 381 in the placebo group; mean age, 48.5 years; 59.6% men) were analyzed, and during the first 2 years, high blood pressure was significantly decreased in patients in the ARB-treated group compared with the placebo-treated group (53 vs. 154 participants; relative risk reduction: 66.3%; $P < 0.001$). Interestingly, after 4 years, high blood pressure was still reduced in patients in the ARB-treated group compared with the placebo-treated group (208 vs. 240 participants; relative risk reduction: 15.6%; $P < 0.007$) during the 2 years of treatment with the placebo. These results also suggest a persistent effect of renin-angiotensin system blockade on the development of hypertension, and early intervention with a tailored medicine will delay the progression of high blood pressure from the public health perspective.

When genome analysis is able to predict the disease probability in the future, the reality of tailored medicine will be achieved. Since the main objective of vaccine therapy is a long-lasting therapeutic effect, it represents a valuable tool to realize the development of tailored medicine.

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