Review Article

Immunological Approaches for Healthy Longevity

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Abstract

As the body ages, the function of various organs changes, bringing sensory expression of “decline” into use, but the background of this phenomenon suggests a role for factors such as cumulative oxidative stress, hypoxia associated with circulatory failure, and lipid peroxidation. Age-related changes and aging are not related directly to passage of time, and the extent of changes differs depending on environmental and individual factors. Practice of Anti-Aging Medicine to confront changes in organ function addressed collectively as “decline” requires care cognizant of functional changes at the cellular level, in other words, an immunological approach. Immunology factors into a wide range of areas, including infection, inflammation, onset, neurology, endocrinology, and regeneration, and in this light, immunological approaches are already in use in a large number of familiar contexts. Aging is accompanied by phenomena including modulation of inflammatory response, immune system modulation for self-defense, changes in reproductive function, and changes in gene expression, for which we seek greater understanding of mechanisms and development of distinctive, corresponding treatment. Recent, more detailed understanding of the mechanisms of natural immunity and acquired immunity has furthered disease prevention through immune control. In this context, we discuss control of the induction process for leukemia associated with retroviral infection, allergen immunotherapy for allergies, and preventive vaccination for Herpes zoster; we likewise introduce recent findings concerning genetic-, molecular-, and individual-level evaluation of the immunosenescence mechanism, which leads to frailty with aging.

KEY WORDS: virus infection, allergy, immune response, immuno therapy, immunosenescence

1) Aging and immunity to viral infections

Some individuals infected with the human T-lymphotropic virus type-1or the hepatitis B or C virus contract leukemia or hepatoma after a latency period of 10 years or longer. This phenomenon is attributed to the induction of multistage genetic changes of somatic cells in the presence of latently infected virus, which ultimately causes tumorigenic transformation of the infected cells, and represents the fact that cancer develops with aging. Because virally-infected cells are efficiently recognized by the host immune system and become targets of elimination by cytotoxic T-lymphocytes (CTL) 1), persistent viral infection and occurrence of consequent oncogenesis requires some means for infected cells to evade elimination by the host immune response. The Epstein-Barr (EB) virus, for example, can immortalize human B-lymphocytes in vitro, and when the resultant lymphoblastic cells are transplanted into an immunocompromized subject such as athymic nude mice , tumors develop, while lymphoma does not emerge in immunocompetent host even when EB virus-induced lymphoblastoid cells are introduced into the body. Lymphomas associated with EB virus is frequently observed in Japanese individuals with AIDS, and the same is true in immunocompromised states accompanying therapy with anticancer agents. The existence of such opportunistic tumors clearly illustrates that decreased immune functions due to aging is linked to occurrence of tumors.

Friend leukemia retrovirus (FV) persistently infects adult mice with a complete immune system and acutely induces fatal leukemia through the accumulation of genetic changes associated with repeated proviral integrations 2). Induction of tumors by retroviral infection generally requires inoculation of newborn mice to induce immunological tolerance to the viral antigens, which allows sustained viremia. In contrast, FV can cause massive splenomegaly followed by fatal leukemia even when inoculated into immuno competent adult mice (Fig. 1). This unique capability is attributed to the fact that FV is a complex
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of two retroviruses: Friend murine leukemia virus (F-MuLV), which is replication-competent retrovirus serving as a helper virus for the other component, and spleen focus-forming virus (SFFV), which is replication-defective but is capable of acutely inducing proliferation of infected erythroblasts F-MuLV alone cannot induce leukemia in adult mice as this virus is rapidly eliminated by host immune responses 2), and thus induction of leukemia with F-MuLV alone requires inoculation of newborn animals. It is, therefore, SFFV that is responsible for immune evasion and the establishment of persistent infection with FV in immunocompetent adult mice.

Substantial differences between mouse strains have been observed with respect to incidence and severity of acute splenomegaly and onset and mortality of leukemia observed after inoculation of adult animals with FV 2,3). Host factors known to affect the process of FV-induced leukemogenesis include factors directly controlling the replication of F-MuLV (e.g., intracellular replication-restricting factors, polymorphisms in viral receptors on cell surfaces, or factors that can bind to such receptors and inhibit particle adhesion), factors controlling SFFV-induced induction of erythroblast replication, and factors controlling host immune response to viral antigens 2,3). We recently revealed that mouse locus encoding APOBEC3, a cytidine deaminase that targets single-stranded DNA and serves as an intracellular, retrovirus-restricting enzyme, is polymorphic, and shows different functionalities among mouse strains. Thus, BALB/c and A strains of mice exhibit low expression levels of APOBEC3 mRNA and protein, and possess the N-terminal amino acid sequence that results in lower function of the above enzyme in comparison with that in C57BL/6 (B6) mice. These strains of mice with less functional APOBEC3 exhibit accelerated F-MuLV replication and retarded production of virus-neutralizing antibodies 4,5). When APOBEC3 is deleted from FV-resistant B6 mice, F-MuLV infects a large proportion of B-lymphocytes, resulting in polyclonal activation of infected B cells and massive reduction in the number of transitional B-lymphocytes that migrate from the bone marrow to secondary lymphoid organs. As a result, FV complex indirectly inhibits the production of virus-neutralizing antibodies 5). B-lymphocytes infected with F-MuLV in the absence of APOBEC3 exhibit the RAE-1 molecule probably due to DNA damage, and become susceptible to killing by natural killer (NK) cells. This may explain the observed reduction of transitional B-lymphocytes upon FV infection of APOBEC3-deficient mice 6). In this way, mouse strains with low APOBEC3 function show high incidence of FV-induced leukemia, as well as high rates of mortality 2-4).

FV-infected cells also have a mechanism for evading the cellular immune response of the host. The incidence and mortality of leukemia after FV infection differ greatly depending on genotypes at the MHC loci, which controls antigen recognition by T-lymphocytes 2,3). We described polymorphic MHC alleles associated with resistance or susceptibility to FV infection, and have identified FV-encoded antigenic epitopes that are recognized by CD4-positive helper T-lymphocytes or CD8-positive CTL 2,7-9). We further examined the possible protective role that T lymphocytes specific for each epitope plays in controlling the onset of leukemia 9-12). The results showed, surprisingly, that the presence of CTL was not essential for Fig. 1. Onset mechanism of Friend Leukemia

Replication-deficient SFFV codes gp55, which binds with erythropoietin receptors and short-form Stk (Sf-Stk), a hematopoietic cell-specific tyrosine phosphorylation receptor, and delivers replication signals to erythroblasts. Chromosomal recombination of F-MuLV or SFFV occurs in the target cells thus added, leading to onset of fatal leukemia mediated by activation of Spi-1, Fli-1, or other oncogenes, or by inactivation of the p53 tumor suppressor gene.
controlling FV-induced leukemia\textsuperscript{11,12}, and that viral antigen-specific CTL rapidly become dysfunctional soon after FV infection\textsuperscript{13}.

In FV-infected mice, expression of PD-1 and a number of other senescence-associated marker molecules on the surface of viral antigen-specific CTL is induced beginning at the very short interval of two weeks after viral inoculation\textsuperscript{13}. Consequently, despite storage of a large amount of granzyme B and other effector molecules in the cytoplasm, FV antigen-specific CTL are not reactivated by restimulation with viral antigens and lack the ability to kill virus-infected target cells \textit{in vitro}\textsuperscript{13}. In fact, FV-infected cells express a PD-1 ligand on their surfaces and evade elimination through the induction of premature senescence of CD8-positive effector T cells by delivering an inhibitory signal through PD-1. Consequently, the process of leukemia induction after FV infection can be controlled by antibody-mediated blocking of signals through PD-1 or other inhibitory receptors (\textit{Fig. 2})\textsuperscript{13,14}. However, to control the onset of leukemia by blocking the above inhibitory signal pathways, PD-1 functions must be blocked soon after FV infection; if the blocking is started at 2 weeks after FV infection, CTL dysfunction cannot be restored\textsuperscript{13}.

The above findings indicate that a mechanism to escape the host’s immune response is involved in virus-induced oncogenesis, which may be prevented by controlling aging of the immune system.

\section*{2) Prospects for allergen immunotherapy}

Allergic rhinitis, an allergic disease, is a classic type I allergic disease governed by antigen-specific onset. Consequently, only allergen immunotherapy (subcutaneous immunotherapy (SCIT) desensitization therapy) is recognized as a curative treatment with antigen specificity having a therapeutic effect during the inductive phase involved in allergic sensitization. SCIT is a therapeutic modality with a 100-year history dating from the 1911 \textit{Lancet} report on grass pollen by Noon, L\textsuperscript{15}, and its therapeutic effect has also been established in double-blind comparative studies. Regrettably, however, SCIT is currently provided for allergic rhinitis only in a limited number of facilities in Japan, probably for considerations of anaphylaxis and other adverse effects, and is not as popular as in the EU and US. Safe, new modalities of immunotherapy are therefore of interest. Another avenue of research is short-duration immunotherapy directed toward cure of allergic rhinitis through several injections.

\subsection*{Current allergen immunotherapy}

In the field of otorhinolaryngology, [SCIT] has demonstrated an 80\% subjective efficacy against house dust (HD) and mites, even on an international basis, and has shown 90\% or greater efficacy for treatment of ragweed allergies in the US\textsuperscript{16}. Even accounting for seasonal variations in its effect against cedar pollinosis in Japan, efficacy is 70\% and is increasingly higher. Post-treatment effects have also been noted internationally\textsuperscript{17}. A 2002 study of cedar pollinosis using the Japanese Rhino-conjunctivitis Quality of Life Questionnaire (JRQLQ) No. 1 also showed better quality of life in an allergen immunotherapy cohort than a drug treatment cohort during the pollen dispersal season (\textit{Fig. 3}).

In the field of internal medicine, the efficacy [of SCIT] is reported as 80-90\% against moderate or less severe atopic asthma\textsuperscript{18,19}. Efficacy against occupational allergies has also been reported. In the field of dermatology, SCIT is generally regarded

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure2.png}
\caption{Cytotoxic T lymphocyte function in FV-induced leukemic onset}
\end{figure}

Even if CD8-positive T cells are eliminated (\(\alpha\)-CD8) from FV-infected mice, the associated leukemic onset process is not substantially affected, and spleen weight increases only slightly (depletable functionality). In other words, the originally CD8-positive T cells in FV-infected mice remain dysfunctional. When the PD-1 and Tim-3 signaling pathways normally inhibiting premature senescence of CD8-positive effector T cells are blocked (\(\alpha\)-PD-1+\(\alpha\)-Tim-3), onset of splenomegaly can be inhibited almost completely. These phenomena indicate that FV infection accompanied by premature senescence causes dysfunction of CD8-positive T-cells (FV-induced exhaustion).
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Fig. 3. QOL scores in drug therapy and allergen immunotherapy for cedar pollinosis

Change in QOL from sublingual immunotherapy (Endpoint value - baseline value)

Placebo group: 21 cases
Investigational drug group: 35 cases

Fig. 4. Change in QOL from sublingual immunotherapy (from Reference 22)

Sublingual allergen immunotherapy

After receiving Ethics Review Board approval for sublingual immunotherapy (SLIT) for cedar pollinosis as currently practiced in the EU and US, we began such practice in 1999.22) When administration is initiated, the concentration of antigen extracts is set at 2 JAU/ml, and concentration is increased daily in stepwise fashion until a maintenance level of 2000 JAU/ml is reached. Administration is performed by adding 1 to 20 drops (approximately 50 µL to 1 ml) of the prepared extract to a small piece of bread (roughly a 1.5 cm square), placing this

as ineffective and even guidelines do not address this modality as a standard therapy. Outside Japan however, efficacy is reported. In pediatrics, there is a high level of evidence for efficacy in both bronchial asthma and allergic rhinitis.20) There are also data showing that SCIT inhibits onset of asthma in pediatric pollinosis.21) However, children reportedly have a higher incidence of anaphylactoid exaggerated immune response than adults, and care is required.
under the tongue for 2 minutes, and then removing the bread. Maintenance is provided as 20 drops of 2000 JAU/ml antigen extract administered sublingually 1×/week. Though the literature provides detailed results, deterioration of QOL was found to have been reduced to half that associated with placebo (Fig. 4)22,23).

Progress in immunological research and associated technologies has already established alternative paths of immunotherapy in the EU, where it is widely used clinically. The methodology of treatment based on the hygiene hypothesis is also becoming better established, and its association with age should also be considered. Theoretically, combination of antigens and adjuvants in particular may come to represent the optimal treatment for allergic diseases. These therapeutic modalities may in the future be used to cure a number of allergic diseases, and primarily pollinosis, without regard to age. But at present, clinical use among humans remains low, and considerable study is required.

3) Herpes zoster and preventive vaccination for Herpes zoster

After a pediatric outbreak of varicella infection, the Varicella zoster virus (VZV) lies dormant in a ganglion and can cause shingles when triggered by stress or some other impetus. Shingles is an age-related disease. Among 48,388 shingles patients in Miyazaki Prefecture, the incidence of onset was 4.15/1000 persons annually, with the rate of onset increasing to 5.8/1000 persons at age 50 years or older, and by age 80 years, approximately 40% had experienced shingles (Fig. 5). Shingles has also increased in other reports, and Miyazaki Prefecture also saw an increase greater than 20% in these 10 years, with a notable increase among females age 60 years and older24).

Herpes zoster is caused by the reactivation of a dormant virus in the trigeminal ganglia, dorsal root ganglia, or other afferent ganglion and migrates to the skin, damaging nerve bundles in the process. At such time, the pain associated with nerve damage is distinctive. In 75% of cases, prodromal pain proceeds eruption of a shingles rash by 3-5 days, and at age 50 years or older, pain continues even after healing of eruptions in a majority of cases. Postherpetic neuralgia (PHN) occurs in 9-19% of shingles patients, at a low risk of 2% in those less than age 50 years, at 20% among those greater than age 50 years, and at approximately 35% among those greater than age 80 years25).

Based on 15 years of Herpes zoster epidemiology among 3500 community residents, Hope-Simpson observed that shingles declines when varicella is prevalent26). A subsequent report stated that shingles was infrequent among adults exposed occupationally to varicella patients. In the US state of Massachusetts, the incidence of varicella was reduced by 79%, from 16.5/1000 person-years to 3.5/1000, over the period of 1998 to 2003 by introduction of routine vaccination with the live Oka varicella vaccine, but the incidence of herpes zoster increased by 90%, from 2.77/1000 to 5.25/100027). This finding suggests that exposure to varicella can boost varicella immunity and prevent herpes zoster. Conversely, the absence of epidemic varicella was found to increase the incidence of herpes zoster in a cohort in which opportunities for immunoboosting were reduced.

On this basis, Oka varicella vaccine was used to prevent varicella, and attempts were made to boost immunity to the VZV and prevent shingles by inoculating adults and elderly individuals with Varicella vaccine. In a large-scale clinical trial among approximately 40,000 adult, elderly subjects age 60 years or older, Oxman et al. reported that Zostavax (a Varicella vaccine) reduced onset of shingles to roughly one-half, and PHN to approximately one-third28). Inoculation of 22,439 adult subjects age 50-59 years with Zostavax was also found to reduce shingles by approximately 70% in a 1-year or longer observational period. Based on these results, Zostavax was found to be indicated in the
US for prevention of shingles among individuals age 50 or older.

While inoculation with measles/rubella vaccines can prevent measles and rubella almost entirely, Zostavax prevents shingles roughly to a half, but onset still occurs half despite vaccination. Considering the fact that the effect of anti-herpes drugs used to treat shingles is to possibly shorten the treatment interval by 1 day, the reduction of incidence to a half is a significantly more beneficial effect than antiviral therapy. However, patients should be advised that measles and rubella may occur even with vaccination in a milder form than without vaccination.

Though the Varicella vaccine used in Japan is deemed to have an immunostimulatory activity for VZV, this vaccine is presently not licensed for prevention of shingles.

Finally, both the Japanese Varicella vaccine and Zostavax for prevention of shingles were manufactured within 10 generations from the original Oka strain of the Varicella vaccine developed in Japan, and the vaccine strains are regarded as the same in immunogenicity or other such properties. The Varicella vaccine used in Japan also has a titer equivalent to that of Zostavax. Consequently, commercial Varicella vaccines are presently not licensed but are deemed to provide a shingles-preventive effect equivalent to that of Zostavax.

4) Immunosuppressant research from the Zizimin2 gene perspective

Techniques for comparison of young and elderly strata have attracted recent attention to research on mechanisms of fragile immunity as an age-related change in biological functions at the cellular level, primarily in lymphocytes, and at the individual level in mice and humans. Just as in the nervous and endocrine systems, immunocompetence is also known to decline with aging and senescence, and changes in acquired immunity, primarily among lymphocytes, are regarded as intimately related to rates of contraction and the progress and prognosis for many diseases that involve the elderly.

Under a mission of “Understanding immunosenescence mechanisms”, we are therefore pursuing research to investigate changes in cell populations and gene expression specific to various immune systems in the body; understand fluctuations in cytokines and other endocrine proteins and the associated mechanisms; maintain immunocompetence in the elderly; standardize levels of immunosenescence to infectious disease, even preventively; and improve age-related changes in gut immunity and body defense functions through perspectives including nutritional intervention.

In this paper, we introduce one aspect of immunosenescence research at this molecular level. We also report on Zizimin2/Dock11 molecular level function of the novel guanine nucleotide exchange factor (GEF), and we report a functional analysis and other information relating to acquired immune system senescence and infectious disease at the individual level, in research using Zizimin2 gene knockout mice.

Zizimin2 (Ziz2) is of interest as an immunosenescence-related gene isolated and identified from B cells in the germinal center of the mouse spleen. It belongs to the Zizimin family, a CDM (CED-5, DOCK180, Myoblast city) family 29-31), and was shown to function as a guanine nucleotide exchange factor which binds to and activates Rho family GTPase and cdc42 through a CZH2 domain alone, was a result suggesting that migration to the cell vicinity of the cell membrane, including migration of these cells and on cell membranes. The fact that migration to the vicinity of the cell membrane, including migration of these projections, was not observed in forced expression of the CZH2 domain alone, was a result suggesting that migration to the cell membrane involves binding with Cdc42 and function of non-CZH2 domain portions having activated domains 33. 

To express Ziz2 by anti-FcγRII/III and LPS stimulation in dendrite cells, we first cultured bone marrow cells for 8 days in the presence of mGM-CSF to induce bone marrow-derived cells (BMDC). On Day 8, Western blotting was used to compare expression of Ziz2 after stimulation of cells with anti-FcγRII/III antigens (2.4G2) and anti-rat IgG Fab', LPS, or anti-CD40. The results showed that transient expression of Ziz2 increased when BMDC were activated by anti-FcγRII/III antibodies or LPA and at the same time ascertained transient activation of intrinsic GTP-type cdc42. These results in BMDC correspond closely to the fact that Ziz2 is present downstream from TLR4 or FcγR signaling.

Ziz2 gene knockout mice were also produced to elaborate age-related and other changes in response to infection from the standpoint of their role in natural immune systems and its bearing on clinical research. In the future, we hope to use these mice for in vivo analysis intended to create an initial understanding of age-related weakening of immune response.

Conclusion

What began with calls in the 1970s for recognition of an aging society became a truly aging society in 2008, when 1 5 individuals was elderly, and 1 10 individuals was highly elderly. Fifty years from now, by some calculations, 1 2.5 individuals will be elderly. Anti-Aging Medicine is by no means intended to inhibit aging itself. As the perspective of the Study Committee for Revision of Senior Health Maintenance Initiatives (October 2004) indicated in its phrase “From a 'Healthy 65' to an 'Active 85', the need is to envision "a new image of the elderly allowing active, meaningful self-fulfillment even while living with illness" and whenever possible, it is crucial to raise the number of “mobile 85-year-olds” engaged in healthy longevity. This translates, in other words, to improved QOL among the elderly and treatment (including prevention) for age-related diseases.

Here we introduce a variety of approaches. Therapies specifically controlling just some pathologies through understanding of molecular mechanisms, control of immune response, immunosuppression, immune strengthening, and other such approaches represent safe and effective, appealing
therapeutic modalities. Though immune mechanisms are acquired by higher animals to distinguish between self and non-self and protect the body from infection, recognition of self versus non-self can then become a trigger for autoimmune disease. In general, the body defense functions of elderly individuals present many problems with regard to monitoring of non-self and tolerance of self, and maintenance of that balance, i.e., homeostasis is all-important. With great expectations, we look forward to future developments in research and results from clinical application.

**Conflicts of Interest:**

The authors declare no financial or other conflicts of interest in the writing of this paper.