Cancer and IgE

Manuel L. Penichet · Erika Jensen-Jarolim
Editors

Cancer and IgE

Introducing the Concept of AllergoOncology

Humana Press

Editors
Manuel L. Penichet
Associate Professor
Division of Surgical Oncology,
Department of Surgery,
and Department of Microbiology,
Immunology, and Molecular Genetics
David Geffen School of Medicine
University of California
at Los Angeles (UCLA)
10833 Le Conte Avenue
Los Angeles, CA 90095
USA
penichet@mednet.ucla.edu

Erika Jensen-Jarolim
Professor and Chair
Department of Pathophysiology
Center of Pathophysiology, Infectiology
and Immunology Medical University
of Vienna Waehringer G. 18-20,
1090 Vienna, Austria
erika.jensen-jarolim@meduniwien.ac.at

ISBN 978-1-60761-450-0 e-ISBN 978-1-60761-451-7
DOI 10.1007/978-1-60761-451-7
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2009939537

Cover illustration: The logo of AllergoOncology created by Dr. Erika Jensen-Jarolim and Mr. Walter Jarolim to promote the first "*1st International AllergoOncology Symposium*," which took place in Vienna, Austria, on April 16, 2007.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Contents

Contributors

Gernot Achatz Department of Molecular Biology, University of Salzburg, Salzburg, Austria

Gertrude Achatz-Straussberger Department of Molecular Biology, University of Salzburg, Salzburg, Austria

Wendy Cozen Departments of Preventive Medicine and Pathology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

Maria A. Curotto de Lafaille Molecular Pathogenesis Program, The Kimmel Center for Biology and Medicine of the Skirball Institute and Department of Pathology, New York University School of Medicine, New York, NY, USA

Tracy R. Daniels Division of Surgical Oncology, Department of Surgery, University of California, Los Angeles (UCLA), Los Angeles, CA, USA

Victor C. de Vries Department of Microbiology and Immunology, Dartmouth Medical School, Lebanon, NH, USA

Julia I. Ellyard Division of Immunology and Genetics, John Curtin School of Medical Research, Australian National University, Canberra, ACT, Australia

Stefan Feichtner Department of Molecular Biology, University of Salzburg, Salzburg, Austria

Hannah J. Gould Randall Division of Cell and Molecular Biophysics, King's College London, Guy's Campus, London, UK

Gustavo Helguera Division of Surgical Oncology, Department of Surgery, University of California, Los Angeles (UCLA), Los Angeles, CA, USA

Erika Jensen-Jarolim IPP – Department of Pathophysiology, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

Sophia N. Karagiannis NIHR Biomedical Research Centre at Guy's and St. Thomas's Hospitals and King's College London, Cutaneous Medicine and Immunotherapy, St. John's Institute of Dermatology, Division of Genetics and

Molecular Medicine, King's College London School of Medicine, Guy's Hospital, London, UK

Sebastian Koenigsberger Department of Molecular Biology, University of Salzburg, Salzburg, Austria

Juan J. Lafaille Molecular Pathogenesis Program, The Kimmel Center for Biology and Medicine of the Skirball Institute and Department of Pathology, New York University School of Medicine, New York, NY, USA

Marinus Lamers Max Planck Institute for Immunobiology, Freiburg, Germany

Stefan Lenz Department of Molecular Biology, University of Salzburg, Salzburg, Austria

Otoniel Martínez-Maza Departments of Obstetrics and Gynecology and Department of Microbiology, Immunology, and Molecular Genetics, Jonsson Comprehensive Cancer Center, AIDS Institute, David Geffen School of Medicine, University of California, Los Angeles (UCLA), Los Angeles, CA, USA

Diana Mechtcheriakova IPP – Department of Pathophysiology, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

Alan Duane Moreno Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

Frank O. Nestle NIHR Biomedical Research Centre at Guy's and St. Thomas's Hospitals and King's College London, Cutaneous Medicine and Immunotherapy, St. John's Institute of Dermatology, Division of Genetics and Molecular Medicine, King's College London School of Medicine, Guy's Hospital, London, UK

Elisa A. Nigro Department of Biology and Genetics, University of Milan and San Raffaele Scientific Institute, Milan, Italy

Randolph J. Noelle Department of Microbiology and Immunology, Dartmouth Medical School, Lebanon, NH, USA

Elizabeth C. Nowak Department of Microbiology and Immunology, Dartmouth Medical School, Lebanon, NH, USA

Elizabeth Ortiz-Sánchez Division of Surgical Oncology, Department of Surgery, University of California, Los Angeles (UCLA), Los Angeles, CA, USA

Isabella Pali-Schoell IPP – Department of Pathophysiology, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

Christopher R. Parish Division of Immunology and Genetics, John Curtin School of Medical Research, Australian National University, Canberra, ACT, Australia

Doris Peckl-Schmid Department of Molecular Biology, University of Salzburg, Salzburg, Austria

Manuel L. Penichet Division of Surgical Oncology, Department of Surgery and Department of Microbiology, Immunology, and Molecular Genetics, Molecular Biology Institute, Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California, Los Angeles (UCLA), Los Angeles, CA, USA

José A. Rodríguez Division of Surgical Oncology, Department of Surgery and Molecular Biology Institute, University of California, Los Angeles (UCLA), Los Angeles, CA, USA

Antonio G. Siccardi Department of Biology and Genetics, University of Milan and San Raffaele Scientific Institute, Milan, Italy

Ljubov Simson Centre for Biomedical, Molecular and Chemical Research, University of Canberra, ACT, Australia

Michelle C. Turner McLaughlin Centre for Population Health Risk Assessment, Institute of Population Health, Faculty of Graduate and Post-Graduate Studies, University of Ottawa, Ottawa, ONT, Canada

Luca Vangelista Department of Biology and Genetics, University of Milan and San Raffaele Scientific Institute, Milan, Italy

Anna Wasiuk Department of Microbiology and Immunology, Dartmouth Medical School, Lebanon, NH, USA

Nadja Zaborsky Department of Molecular Biology, University of Salzburg, Salzburg, Austria

Chapter 1
Introduction

Erika Jensen-Jarolim and Manuel L. Penichet

1.1 Background

Infectious diseases, being the major burden in the history of mankind worldwide until the beginning of the 20th century, were important triggers in the understanding of immunological mechanisms. In contrast to infectious diseases, reports of allergies and cancers were less common, but increased tremendously within the last century. Based on the US mortality data of the National Center for Health Statistics, Centers for Disease Control and Prevention 2009, a recent report from the American Cancer Society indicated that the number of cancer deaths increased approximately from 100,000 to 550,000 per year between 1930 and 2006, paralleling the increase of the total population during this period. Leading causes of death from cancer are lung and bronchus cancer, in men prostate cancer, and in women breast cancer [1, 2]. Normalization to population size shows that the cancer death rate for most malignancies has been generally stable, although the mortality rate of certain malignancies, such as lung and bronchus cancer, has increased over the last 50 years [1-3].

In allergy, the situation is less clear, because for the time period around the turn of the 19th century, only imprecise information is available. However, within the last 30 years the incidences of allergies has doubled not only in industrial countries, but in developing countries as well [4]. From the diagnosed atopic state it is concluded that in some areas, such as in New Zealand, the incidence of skin and respiratory allergies increased as much as 40% in the population [5]. Thus, the cancer and allergy incidence parallel each other, whereas infectious diseases have decreased in industrialized countries. From an epidemiological point of view, the decreased parasite and bacterial burden may indirectly correlate with the increasing curves of allergy and cancer, being thus in accordance with the so-called hygiene hypothesis. This theory links the recent lower parasite and microbe burden to aberrant immune responses or failure of immune mechanisms [6, 7]. However, if allergies and malignancies both

E. Jensen-Jarolim (✉)
IPP-Department of Pathophysiology, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria
e-mail: erika.jensen-jarolim@meduniwien.ac.at

M.L. Penichet, E. Jensen-Jarolim (eds.), *Cancer and IgE*,
DOI 10.1007/978-1-60761-451-7_1,

increase, does that mean a linked positive association? This question is difficult to answer with epidemiology, as both diseases are correlated with a large number of genetic and environmental causative factors which again change continuously [8].

Based on clinical suspicion, multiple epidemiological studies have so far been undertaken to examine whether any, positive or negative association of allergies and cancer exist. Significant *p*-values demonstrating the inverse association between the incidence of allergies and/or levels of IgE and malignancies have been reported in several studies including a recent cohort study with a sample size above 1 million investigated persons [9, 10]. These results suggest a potential role of IgE in cancer immunosurveillance at least in certain tumors. Chapters 4 and 5 of this book give a comprehensive overview on these epidemiological approaches.

Although statistical methods are very helpful for capturing interrelationships, more experimental evidence is needed to find a cause and effect relationship. After evaluation of first tendencies by cohort studies, case–control studies and experimental in vitro and in vivo data must follow to achieve evidence-based medical statistics. Dependent on the development of modern laboratory technologies, knowledge about the immunological and molecular mechanisms of allergies and malignancies can be acquired. Interestingly, during the last decades a number of experimental approaches were developed to investigate whether factors and cells participating in the pathophysiology of allergy also are players in cancer. Let us move again one step back to history.

1.2 History of Allergy

Pioneers in medical history like John Bostock realized and were concerned about the increasing cases of hay fever from the beginning of the 19th century on, and Charles Bakely in 1869 invented skin testing as a diagnostic tool for specific sensitization [11]. However, much more focus was given to the major threats of this period, which were infectious diseases. Besides the continuous development of active immunization, passive applications of antisera raised in animals like horses were standard treatment options for infected patients. The so-called antitoxins were the first major success of therapeutic immunology, representing in fact specific immunoglobulins. Soon the risk of anaphylactic side reactions upon repetitive injection of antisera was recognized and feared: the death rates in 1890 were still 60%, in 1894 30%, and, after improvement of the industrial manufacturing of antitoxins, e.g., by ammonium precipitation, still in 1910 10% of the treated patients died as reported by the Metropolitan Asylums Board 1895–1910 [12]. During this time, Charles R. Richet and Paul Portier succeeded in imitating this immediate type hypersensitivity reaction by immunizing dogs with a protein extract of sea anemones (Actinaria). To describe this immediate type inflammatory reaction provoked by a specific antigen (which was considered to be a toxin), Richet and Portier coined the term anaphylaxis in 1902 [13]. The

word anaphylaxis is derived from the Greek words *ana* (“against”) and *phylaxis* (“protection”).

The pediatricians Clemens Freiherr von Pirquet from Vienna and Béla Schick, a Hungarian, were concerned about these sudden (anaphylactic) reactions and realized that by skin testing with the antiserum the (hyper) immune state of the patient could be predicted. This cognition led to the development of the Schick test for diphtheria and the Pirquet test for tuberculosis, routine tests which are still applied worldwide. Moreover, Pirquet eternized himself by coining the term “allergy” from the Greek words *allos* (“other”) and *ergon* (“reaction”) [14]. Thereby, he meant sensitization leading to hypersensitivity against toxins or infectious agents. Thus, the term “allergy” was in the beginning not associated with exogenous innocuous antigens like pollen or house dust mite. It took decades until it was understood that the key mechanism for “Pirquet’s allergy” also accounts for allergy in its recent sense. And it took even longer until the key players were identified.

Focusing on a typical allergen, namely fish protein, Prausnitz and Küstner described in 1921 that a soluble, transferable “skin sensitizing factor” is responsible for hypersensitivities [15], which was shortly later nominated “atopic reagin” by Arthur F. Coca and Ella F. Grove [16]. These discoveries triggered research on “reagins” during the following five decades, analyzing their distribution [17], demonstrating their specificity for allergens [18, 19] and helminth antigens [20–22], attempting to purify them [23], and identifying them as a prototype of cytophilic antibodies, i.e., antibodies preferentially fixed to cells in contrast to free antibodies [24]. Coincidently, eosinophilic and basophilic granulocytes were detected in atopic skin [25].

The simultaneous discovery of IgE myelomas in two independent laboratories [26–29], closely followed by a third [30] identified reagins as being the fifth class of immunoglobulins with a key role in the pathophysiology of immediate type hypersensitivity. Finally, the detection of high- and low-affinity IgE receptors, being the last missing link between the effector cells and IgE antibodies became the explanatory mechanism of the anaphylactic reaction [31–34]. The molecular events restraining potentially dangerous high serum levels of IgE is reviewed in Chapter 2, the high specification of the IgE maturation program in Chapter 3, and the interplay of secreted IgE with its receptors determining its functional properties in Chapters 7 and 8.

The mechanism of the anaphylactic reaction is thus a consequence of allergens crosslinking IgE bound to its high-affinity receptor. Thereby, molecular patterns displayed by the allergens seem to be important and could be a common feature of allergenic molecules (Chapter 10). The released mediators as a consequence of the anaphylactic reaction have strong proinflammatory effects and are possibly effective in worm expulsion, but clearly detrimental in allergy when harmless allergens can produce mild to severe anaphylactic reactions in sensitized patients. Thus, it is accepted today that elevated specific and/or total IgE and Th2-type responses in general are hallmarks of allergies and parasitic diseases. However, as opposed to allergies, in helminth infection typically no anaphylactic shock is observed [35].

1.3 History of AllergoOncology

It is more or less neglected today that the association of tumor occurrence and allergic inflammation has been discussed since the first half of the twentieth century [36, 37] and continuously ever since (Table 1.1). Possibly the first publication on "Allergy against cancer" stems from E.G. Martin and was published in 1935 [36]. He investigated why "one person will develop carcinoma, while another does not," and his unanswered question was whether "the conception of allergy or idiosyncrasy could be broadened to include this mysterious fact?" Well, one has to admit that his usage of the term "allergy" was related to a rather imprecise scientific and clinical definition at that time. Still, in 1952 the view had not changed much when Bienengraber investigated "Tumor metastasis in the light of allergology" [37]. He analyzed the stages of metastasis and found that individually different dynamics of vascularization and transudation could be observed during the nesting phase of the tumor cells. Dependent on the sensitization state of the patient he categorized normergic, hypergic, anergic, and eu-ergic reactions. He found that these observations in tumor tissues paralleled the inflammatory reaction seen toward parasites, microbes, and self-antigens. Even though he likely meant "specific inflammation" he termed the phenomenon "tumor allergy".

Table 1.1 AllergoOncology is tightly connected with the history of allergy. This table gives an overview of selected key publications

Authors	Title	Reference
Definition of allergy and era of reagins		*1906*
Von Pirquet	Allergy	[14]
Martin	Predisposing factors and diagnosis of rectal cancer: a discussion of allergy	[36]
Bienengraber	Tumor metastasis in the light of allergology	[37]
Molomut et al.	The effect of an allergic inflammatory response in the tumor bed on the fate of transplanted tumors in mice	[38]
Berdel et al.	Mechanism of tumor allergy and its importance in tumor pathogenesis	[39]
Schlitter	Is there an allergy against malignant tumor tissue and what can it signify in regard to the defense of the body against cancer?	[41]
Identification of IgE		*1967*
Ishizaka and Ishizaka	Identification of gamma-E-antibodies as a carrier of reaginic activity	[27]
Johansson	Raised levels of a new immunoglobulin class (IgND) in asthma	[28]
Ure	Negative association between allergy and cancer	[44]
McCormick et al.	A study of allergy in patients with malignant lymphoma and chronic lymphocytic leukemia	[45]
Augustin and Chandradasa	IgE levels and allergic skin reactions in cancer and non-cancer patients	[46]

Table 1.1 (continued)

Authors	Title	Reference
Nagy et al.	Growth inhibition of murine mammary carcinoma by monoclonal IgE antibodies specific for the mammary tumor virus	[57]
Kershaw et al.	Tumor-specific IgE-mediated inhibition of human colorectal carcinoma xenograft growth	[56]
Gould et al.	Comparison of IgE and IgG antibody-dependent cytotoxicity in vitro and in a SCID mouse xenograft model of ovarian carcinoma	[55]
Reali et al.	IgEs targeted on tumor cells: therapeutic activity and potential in the design of tumor vaccines	[63]
Karagiannis et al.	Activity of human monocytes in IgE antibody-dependent surveillance and killing of ovarian tumor cells	[64]
Turner et al.	Cancer mortality among US men and women with asthma and hay fever	[9]
Riemer et al.	Active induction of tumor-specific IgE antibodies by oral mimotope vaccination	[58]
Definition of AllergoOncology		*2006*
Jensen-Jarolim et al.	AllergoOncology: the role of IgE in tumor defense	[62]
Karagiannis et al.	IgE-antibody-dependent immunotherapy of solid tumors: cytotoxic and phagocytic mechanisms of eradication of ovarian cancer cells	[54]
Bracher et al.	Three-colour flow cytometric method to measure antibody-dependent tumour cell killing by cytotoxicity and phagocytosis	[65]
Fu et al.	Immunoglobulin E antibodies from pancreatic cancer patients mediate antibody-dependent cell-mediated cytotoxicity against pancreatic cancer cells	[51]
Jensen-Jarolim et al.	AllergoOncology: the role of IgE-mediated allergy in cancer	[10]
Karagiannis et al.	Characterisation of an engineered trastuzumab IgE antibody and effector cell mechanisms targeting HER2/*neu*-positive tumour cells	[66]

The concept was, however, taken up again in the 1950s, when Molomut in a pioneer study constructed an in vivo model for examining the "allergic response" toward tumors [38]. He applied tumor transplants in two different strains of ovalbumin-sensitized inbred mice, fibrosarcoma S621 in C/Scott mice, and sarcoma I in DBA1/Jax mice. In this large experimental setup allergen challenges at the tumor sites could not prevent the growth of the transplants and he concluded "...that the localized allergic inflammatory response at the site of a tumor implantation per se has no effect upon the development of the graft." However, there might have been several sources of error in his experiment: (1) the transplanted tumor cells were driven by a dominant oncogene signal, (2) too many

cells within the "tiny tumor graft" and/or most importantly, and (3) the complete neglect of antigen-specificity. Taken together, we can conclude from this study that antigenic crossreactivity between ovalbumin and fibrosarcoma S621 and the used sarcoma cells does not exist. On the other hand, it should be noted that Molomut in his work used the current definition of the "allergy" term.

In the study entitled "Mechanism of tumor allergy and its importance in tumor pathogenesis" [39], Berdel and coworkers investigated the responses to tumor transplants and reported that upon repetitive injections of tumor cells in mice anaphylaxis in the sense of hyperacute transplant rejection can occur. Simultaneously the authors reported the phenomenon of tolerance when mice with spontaneous tumors are not able to reject tumor transplants. Based on previous observations for tuberculosis and pollen allergy, they concluded that a tumor-specific cytotoxic antibody must be formed in the first phase, followed by a second antibody with a neutralizing effect, later termed a blocking antibody [40]. The authors suggested that antigenic changes of the tumor cells are responsible for the specificity of the events.

Eosinophils were at that time recognized as a hallmark of allergic and parasitic diseases, and their appearance in tumor tissues had also been noticed. In the publication "Is there an allergy against malignant tumor tissue and what can it signify in regard to the defense of the body against cancer?", H.E. Schlitter compared tumors with parasitic microorganisms and analyzed the association of eosinophilia in the blood and tissues with the formation of metastases [41]. In his review he cites work of K. Ebhardt [42] and W. Fischer 1952 [43] who interpreted the eosinophilia in malignancies as a sign of a "special allergic condition." According to Schlitter's pathological experience tumor patients with hypereosinophilia were typically characterized with a high burden of metastases. On the other hand, he referred to studies in the elderly where too low numbers of eosinophils were disadvantageous and associated with rapid growth of the tumor. Thus, in his view the eosinophil could either be promoting tumor progression or be a regulatory counter-reaction to metastasis.

Shortly after the discovery of IgE in 1967 [27, 28], namely in 1969 D.M. Ure announced a negative association between atopy and gynecologic malignancies [44]. Thereafter, IgE determinations were already included in the following "allergooncological" examinations. For example, the group around Terry and Kimi Ishizaka was performing "A study of allergy in patients with malignant lymphoma and chronic lymphocytic leukemia" [45]. Interestingly, among Hodgkin's disease, reticulum cell sarcoma, lymphosarcoma, and chronic lymphatic leukemia (CLL), only in CLL decreased incidences of allergic symptoms throughout the lives associated with lowered IgE levels were found. Generally, in the lymphoma patients a large number of patients had noted that they lost allergic symptoms with the onset of the malignancy. The authors concluded that CLL patients might have a hereditary immunologic defect becoming apparent only later in life.

The discovery of IgE immunoglobulins made it possible to design more precise studies investigating the relationship of allergy or atopy and cancer [46, 47]. When Jacobs et al. studied circulating IgE levels they found that in 200 untreated cancer patients approximately half had very low levels (20–680 units per ml) and, on the contrary, others had exceptionally high levels (above 100,000 units per ml)

determined by radioimmunoassay but not by radial immunodiffusion. The authors suggested that cancer patients might form certain inhibitor substances from the tumors which interfere with the precipitation of IgE in the radial immunodiffusion assay, the results thus might, "...be considered as indicating the absence of IgE." They observed this phenomenon in sera as well as in blood samples from vessels draining tumors. The identity of this putative factor remains elusive to this day.

Coming back to epidemiology, for example, Joseph Allegra et al. described that there was a 15-fold decrease in prevalence of atopy in the cancer population in a Lancet paper in 1976 [48]. Atopic dermatitis patients also have a lower risk of developing melanoma and this may be due to the enhanced surveillance function of IgE being increasingly bound to the IgE receptors of the skin being overexpresssed in this disease [49].

Finally, Neuchrist et al. were the first who histologically detected natural IgE within tumor tissues of head and neck cancer patients; however, the function of these IgE antibodies was not determined [50]. Surprisingly, IgE was the most abundant and pronounced immunoglobulin isotype and appeared clustered on dendritic-like cells within the epithelial tumor tissues. Only a very recent study re-examined this subject again by isolating natural IgE from the serum of pancreatic carcinoma patients [51]. Indeed, high tumoricidic properties could be demonstrated pointing toward a defence function of these natural antitumor IgE antibodies. Similarly, as in atopic dermatitis where autoreactive IgE against keratinocyte antigens is found [52], anaphylaxis was not reported in any of the pancreatic cancer patients [51]. With respect to the mechanisms of antitumor IgE, antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP) seem to be especially important [53, 54], as described in Chapters 7 and 8. These chapters also discuss a variety of effector cells involved in ADCC and/or ADCP such as basophils, dendritic cells, Langerhans cells, and monocytes/macrophages. In this context, the role of mast cells and eosinophils in cancer, as well as of Th2 immunity in general, is discussed in Chapters 6 and 11, respectively.

Advancements in molecular biology and genetic engineering enabled several groups in the 1990s to design antitumor antibodies also of the IgE class [55–57]. Importantly, IgE antibodies can be used as direct therapeutics in passive immunotherapy of cancer (Chapters 7 and 8) and also as adjuvant of cancer vaccines (Chapter 9). Chapter 7 also describes an alternative strategy based on the induction of endogenous IgE antitumor response based on an oral mimotope vaccination [58]. The recombinant antibodies are not only useful for experimentally studying the effects of IgE-targeting of cancer cells, but they could be useful biologicals in cancer therapy of patients in the future. Undoubtedly, targeted therapy using chimeric, humanized, or fully human IgG antibodies has been a world success [59–61].

However, as discussed in Chapter 7, antibodies of the IgE class have several properties that may be advantageous for cancer therapy such as the fact that IgE binds to its Fc epsilon receptors (FcϵRs) with much higher affinity compared to that of IgG for the Fc gamma receptors (FcγRs), and the very low serum levels of IgE that provide minimal competition for Fc receptor occupancy further facilitating ADCC/ADCP. Therefore, in contrast to IgG, IgE could be applied in lower dosages

reducing the economic costs and increasing the efficacy. In fact, the large total doses of the therapeutics antibodies that are often required have been described as a limitation in their use as drugs [59]. However, it is important to stress that the use of antibodies of the IgG and IgE classes in cancer therapy is not necessarily mutually exclusive, as both classes of antibodies could be used in combination (simultaneously or sequentially) potentially maximizing their efficacy in certain cases through the engagement of different Fc receptors and effector cells.

1.4 Synopsis

So far numerous studies have evaluated the question of whether the same immunological mechanisms could be shared in malignancies and allergic disorders, either promoting or inhibiting disease. Nevertheless, until now a relationship between the two fields has not been officially acknowledged. This may be due to the fact that experts in IgE biology are today home to allergological societies. Vice versa, the oncology scene is dominated today by search for small molecules interfering with signaling, or attempts to activate antitumor cytotoxic T-lymphocytes, or by passive immunotherapy with IgG antibodies. Therefore, we strongly believe that there is a rationale for a platform where allergological and oncological know-how can be exchanged for the benefit of the patient. AllergoOncology can be defined as a field that aims to reveal the function of IgE-mediated immune responses against tumor cells in order to enhance the understanding of its biology and develop novel IgE-based treatment options against malignant diseases.

The term AllergoOncology was first proposed at the 2006 Malta-Meeting of the Collegium Internationale Allergologicum (CIA) [62]. The oral presentation was entitled “AllergoOncology, the role of IgE in tumor defense” and the audience was not ready for this provocation. Professor Johannes Ring, then president of the CIA, subsumed the event in the minutes of the meeting as follows: “*. . .a novel subject was presented at this meeting – AllergoOncology, a topic so hot that the discussion was immediately interrupted after the first question*. . ..”

Encouraged by equally many positive comments during this meeting the story went on [10]. To date, two International AllergoOncology Symposia have taken place in Vienna, and an AllergoOncology task force was officially approved by the World Allergy Organization in 2008, succeeding in implementing the subject into the World Allergy Congress 2009 for the first time. Therefore, we felt it is the right time to venture the first AllergoOncology book.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, and Thun MJ (2009) Cancer Statistics, 2009. CA Cancer J Clin 59:225–49
2. American Cancer Society (2009) American Cancer Statistics 2009. http://www.cancer.org/docroot/STT/STT_0.asp

3. Wingo PA, Cardinez CJ, Landis SH, Greenlee RT, Ries LA, Anderson RN, and Thun MJ (2003) Long-term trends in cancer mortality in the United States, 1930–1998. Cancer 97:3133–275
4. Pearce N and Douwes J (2006) The global epidemiology of asthma in children. Int J Tuberc Lung Dis 10:125–32
5. Sly RM (1999) Changing prevalence of allergic rhinitis and asthma. Ann Allergy Asthma Immunol 82:233–48
6. Strachan D (2000) Family size, infection and atopy: the first decade of the "hygiene hypothesis". Thorax 55:2–10
7. Namazi MR (2007) Extension of the "Hygiene Hypothesis" to the negative association between acne and atopy, hematological malignancies, and malignant melanoma. Medical Hypotheses 69:960–961
8. Karhausen LR (2000) Causation: the elusive grail of epidemiology. Med Health Care Philos 3:59–67
9. Turner MC, Chen Y, Krewski D, Ghadirian P, Thun MJ, and Calle EE (2005) Cancer mortality among US men and women with asthma and hay fever. Am J Epidemiol 162:212–21
10. Jensen-Jarolim E, Achatz G, Turner MC, Karagiannis S, Legrand F, Capron M, Penichet ML, Rodriguez JA, Siccardi AG, Vangelista L, Riemer AB, and Gould H (2008) AllergoOncology: the role of IgE-mediated allergy in cancer. Allergy 63:1255–66
11. Emanuel MB (1988) Hay fever, a post industrial revolution epidemic: a history of its growth during the 19th century. Clinical Allergy 18:295–304
12. Biggs JP (1912) London Evidence Against Anti-Toxin (Chapter 107). In: Sanitation Versus vaccination. Leicester
13. Richet CR and Portier P (1902) De l'action anaphylactique de certain venins. Comptes rendus de la Société de biologie, Paris 54:170–2
14. Pirquet C (1906) Allergy. Münchener Medizinische Wochenschrift 53:1457–8
15. Prausnitz C and Kustner H (1921) Studien über die Ueberempfindlichkeit. Zentralbl Bakteriol 86:160–9.
16. Coca AF and Grove EF (1925) A study of the atopic reagins. J Immunol 10:445–64
17. Scherrer E (1930) The distribution of reagins in the blood plasma. J Allergy 2:467
18. Layton LL and Yamanaka E (1962) Demonstration of human reagins to foods, cat dander, an insect, and ragweed and grass pollens. J Allergy 33:271–5
19. Augustin R (1967) Demonstration of reagins in the serum of allergic subjects. From WEIR, Handbook of experimental immunology Oxford-Edinburgh: Blackwell, p. 1076
20. Kailin EW, Rossbach EA, and Walzer M (1950) Factors influencing reagin formation in experimental human sensitization to ascaris lumbricoides antigen; the influence of a previous sensitization on rate of sensitization. J Allergy 21:225–31
21. Ogilvie BM (1964) Reagin-like antibodies in animals immune to helminth parasites. Nature 204:91–2
22. Zvaifler NJ, Sadun E, and Becker EL (1966) Anaphylactic (reaginic) antibodies in helminthic infections. Clin Res 14:336
23. Vaerman JP, Epstein W, Fudenberg H, and Ishizaka K (1964) Direct demonstration of reagin activity in purified gamma 1a globulin. Nature 203:1046–8
24. Augustin R, Connolly R, and Lloyd G (1964) Atopic reagin as a prototype of cytophilic antibodies. Aus: Protides Biol. Fluids, Proc. 11th Coll. Bruges 1963, p. 56, H. Peeters (ed.). Amsterdam: Elseviers
25. Felarca AB and Lowell FC (1971) The accumulation of eosinophils and basophils at skin sites as related to intensity of skin reactivity and symptoms in atopic disease. J Allergy Clin Immunol 48:125–33
26. Ishizaka K and Ishizaka T (1966) Physicochemical properties of reaginic antibody. 1. Association of reaginic activity with an immunoglobulin other than gammaA- or gammaG-globulin. J Allergy 37:169–85
27. Ishizaka K and Ishizaka T (1967) Identification of gamma-E-antibodies as a carrier of reaginic activity. J Immunol 99:1187–98

28. Johansson SG (1967) Raised levels of a new immunoglobulin class (IgND) in asthma. Lancet 2:951–3
29. Johansson SG and Bennich H (1967) Immunological studies of an atypical (myeloma) immunoglobulin. Immunology 13:381–94
30. Fishkin BG, Orloff N, Scaduto LE, Borucki DT, and Spiegelberg HL (1972) IgE multiple myeloma: a report of the third case. Blood 39:361–7
31. Carson DA and Metzger H (1974) Interaction of IgE with rat basophilic leukemia cells. IV. Antibody-induced redistribution of IgE receptors. J Immunol 113:1271–7
32. Metzger H, Rivnay B, Henkart M, Kanner B, Kinet JP, and Perez-Montfort R (1984) Analysis of the structure and function of the receptor for immunoglobulin E. Mol Immunol 21:1167–73
33. Nakajima T and Delespesse G (1986) IgE receptors on human lymphocytes. I. Identification of the molecules binding to monoclonal anti-Fc epsilon receptor antibodies. Eur J Immunol 16:809–14
34. Ludin C, Hofstetter H, Sarfati M, Levy CA, Suter U, Alaimo D, Kilchherr E, Frost H, and Delespesse G (1987) Cloning and expression of the cDNA coding for a human lymphocyte IgE receptor. Embo J 6:109–14
35. Mangan NE, Fallon RE, Smith P, van Rooijen N, McKenzie AN, and Fallon PG (2004) Helminth infection protects mice from anaphylaxis via IL-10-producing B cells. J Immunol 173:6346–56
36. Martin EG (1935) Predisposing factors and diagnosis of rectal cancer: a discussion of allergy. Ann Surg 102:56–61
37. Bienengraber A (1952) Tumor metastasis in the light of allergology. Zentralbl Chir 77: 1873–81
38. Molomut N, Spain DM, Kreisler L, and Warshaw LJ (1955) The effect of an allergic inflammatory response in the tumor bed on the fate of transplanted tumors in mice. Cancer Res 15:181–3
39. Berdel W, Nass G, and Wiedemann G (1956) Mechanism of tumor allergy and its importance in tumor pathogenesis. Int Arch Allergy Appl Immunol 9:200–21
40. Loveless M and Cann JR (1955) Distribution of blocking antibody in human serum proteins fractionated by electrophoresis-convection. J Immunol 74:329–39
41. Schlitter HE (1961) Is there an allergy against malignant tumor tissue and what can it signify in regard to the defense of the body against cancer? Strahlentherapie 114:203–24
42. Ebhardt K (1937) Mschr. Krebsbekämpfung: 33
43. Fischer W (1952) Zbl. Path. 89:129
44. Ure DM (1969) Negative association between allergy and cancer. Scott Med J 14:51–4
45. McCormick DP, Ammann AJ, Ishizaka K, Miller DG, and Hong R (1971) A study of allergy in patients with malignant lymphoma and chronic lymphocytic leukemia. Cancer 27:93–9
46. Augustin R and Chandradasa KD (1971) IgE levels and allergic skin reactions in cancer and non-cancer patients. Int Arch Allergy Appl Immunol 41:141–3
47. Jacobs D, Landon J, Houri M, and Merrett TG (1972) Circulating levels of immunoglobulin E in patients with cancer. Lancet 2:1059–61
48. Allegra J, Lipton A, Harvey H, Luderer J, Brenner D, Mortel R, Demers L, Gillin M, White D, and Trautlein J (1976) Decreased prevalence of immediate hypersensitivity (atopy) in a cancer population. Cancer Res 36:3225–6
49. Synnerstad I, Fredrikson M, Ternesten-Bratel A, and Rosdahl I (2008) Low risk of melanoma in patients with atopic dermatitis. J Eur Acad Dermatol Venereol 22:1423–8
50. Neuchrist C, Kornfehl J, Grasl M, Lassmann H, Kraft D, Ehrenberger K, and Scheiner O (1994) Distribution of immunoglobulins in squamous cell carcinoma of the head and neck. Int Arch Allergy Immunol 104:97–100
51. Fu SL, Pierre J, Smith-Norowitz TA, Hagler M, Bowne W, Pincus MR, Mueller CM, Zenilman ME, and Bluth MH (2008) Immunoglobulin E antibodies from pancreatic cancer patients mediate antibody-dependent cell-mediated cytotoxicity against pancreatic cancer cells. Clin Exp Immunol 153:401–9

52. Altrichter S, Kriehuber E, Moser J, Valenta R, Kopp T, and Stingl G (2008) Serum IgE autoantibodies target keratinocytes in patients with atopic dermatitis. J Invest Dermatol 128:2232–9
53. Karagiannis SN, Bracher MG, Beavil RL, Beavil AJ, Hunt J, McCloskey N, Thompson RG, East N, Burke F, Sutton BJ, Dombrowicz D, Balkwill FR, and Gould HJ (2008) Role of IgE receptors in IgE antibody-dependent cytotoxicity and phagocytosis of ovarian tumor cells by human monocytic cells. Cancer Immunol Immunother 57:247–63
54. Karagiannis SN, Bracher MG, Hunt J, McCloskey N, Beavil RL, Beavil AJ, Fear DJ, Thompson RG, East N, Burke F, Moore RJ, Dombrowicz DD, Balkwill FR, and Gould HJ (2007) IgE-antibody-dependent immunotherapy of solid tumors: cytotoxic and phagocytic mechanisms of eradication of ovarian cancer cells. J Immunol 179:2832–43
55. Gould HJ, Mackay GA, Karagiannis SN, O'Toole CM, Marsh PJ, Daniel BE, Coney LR, Zurawski VR, Jr, Joseph M, Capron M, Gilbert M, Murphy GF, and Korngold R (1999) Comparison of IgE and IgG antibody-dependent cytotoxicity in vitro and in a SCID mouse xenograft model of ovarian carcinoma. Eur J Immunol 29:3527–37
56. Kershaw MH, Darcy PK, Trapani JA, MacGregor D, and Smyth MJ (1998) Tumor-specific IgE-mediated inhibition of human colorectal carcinoma xenograft growth. Oncol Res 10: 133–42
57. Nagy E, Berczi I, and Sehon AH (1991) Growth inhibition of murine mammary carcinoma by monoclonal IgE antibodies specific for the mammary tumor virus. Cancer Immunol Immunother 34:63–9
58. Riemer AB, Untersmayr E, Knittelfelder R, Duschl A, Pehamberger H, Zielinski CC, Scheiner O, and Jensen–Jarolim E (2007) Active induction of tumor-specific IgE antibodies by oral mimotope vaccination. Cancer Res 67:3406–11
59. Carter PJ (2006) Potent antibody therapeutics by design. Nat Rev Immunol 6:343–57
60. Maggon K (2007) Monoclonal antibody "gold rush". Curr Med Chem 14:1978–87
61. Reichert JM and Valge-Archer VE (2007) Development trends for monoclonal antibody cancer therapeutics. Nat Rev Drug Discov 6:349–56
62. Jensen-Jarolim E, Untersmayr E, Knittelfelder R, Zielinski CC, Scheiner O, Duschl A, and Riemer AB (2006) Allergo-Oncology: the role of IgE in tumor defense. 26th Symposium of the Collegium Internationale Allergologicum. May 5–10, Malta.
63. Reali E, Greiner JW, Corti A, Gould HJ, Bottazzoli F, Paganelli G, Schlom J, and Siccardi AG (2001) IgEs targeted on tumor cells: therapeutic activity and potential in the design of tumor vaccines. Cancer Res 61:5517–22
64. Karagiannis SN, Wang Q, East N, Burke F, Riffard S, Bracher MG, Thompson RG, Durham SR, Schwartz LB, Balkwill FR, and Gould HJ (2003) Activity of human monocytes in IgE antibody-dependent surveillance and killing of ovarian tumor cells. Eur J Immunol 33: 1030–40
65. Bracher M, Gould HJ, Sutton BJ, Dombrowicz D, and Karagiannis SN (2007) Three-colour flow cytometric method to measure antibody-dependent tumour cell killing by cytotoxicity and phagocytosis. J Immunol Methods 323:160–71
66. Karagiannis P, Singer J, Hunt J, Gan SK, Rudman SM, Mechtcheriakova D, Knittelfelder R, Daniels TR, Hobson PS, Beavil AJ, Spicer J, Nestle FO, Penichet ML, Gould HJ, Jensen-Jarolim E, and Karagiannis SN (2009) Characterisation of an engineered trastuzumab IgE antibody and effector cell mechanisms targeting HER2/*neu*-positive tumour cells. Cancer Immunol Immunother 58:915–30

Chapter 2
The Biology of IgE: Molecular Mechanism Restraining Potentially Dangerous High Serum IgE Titres In Vivo

Gernot Achatz, Gertrude Achatz-Straussberger, Stefan Feichtner, Sebastian Koenigsberger, Stefan Lenz, Doris Peckl-Schmid, Nadja Zaborsky, and Marinus Lamers

Abstract Our knowledge about the regulation of the expression of IgE and its biological function is at best limited. We do, however, know that the production of IgE is tightly regulated which is reflected by the fact that the steady-state serum levels of IgE in mice and humans are 3–4 orders of magnitude lower if compared to IgG1, which is an immunoglobulin isotype expressed in response to the same cytokine milieu. What are the rate-limiting steps responsible for this discrepancy? In the following chapter six molecular mechanisms restraining IgE levels will be discussed in detail. The understanding of these mechanisms, combined with the analysis of the biological function of the IgE molecule during an immune response, is the prerequisite for the establishment of new systemic IgE-targeted therapeutic strategies in the future.

2.1 Introduction

IgE is an evolutionary conserved member of the immunoglobulin (Ig) family. Compared to all other Ig classes, which are present in concentrations of micrograms to milligrams per ml serum, the titre of IgE is very low (nano- to micrograms per ml range) in plasma of normal healthy individuals and of normal laboratory mouse strains. IgE is most prominent in epitheliae and mucosae where it is bound to specific receptors on highly potent effector cells like eosinophilic granulocytes and mast cells. Bound to these cells IgE has a long half-life (weeks to months), while free in plasma the half-life is very short (∼6 hours). This suggests that IgE plays a role in local immune defence mechanisms. However, the core function for IgE is still unknown. From an evolutionary point of view, IgE is conserved and can be found in all mammalia, including monotremata [1]. It therefore originated at least 160 million years ago, possibly even more than 300 million years ago [2], from a gene duplication of IgY, in which the anaphylactic and opsonic activities of IgY were separated,

G. Achatz (✉)
Department of Molecular Biology, University of Salzburg, Salzburg, Austria
e-mail: gernot.achatz@sbg.ac.at

M.L. Penichet, E. Jensen-Jarolim (eds.), *Cancer and IgE*,
DOI 10.1007/978-1-60761-451-7_2,

giving rise to IgE and IgG, respectively [3]. Apparently, in an evolutionary sense, anaphylactic defence mechanisms are needed but at a potentially high price to the organism. The division of anaphylactic and opsonic activities in separate genes allowed principally a tighter and more specific control of both immune mechanisms. In these days IgE is best known for its strong, unwanted effector functions, in the form of allergic reactions [4]. These can range from annoying, local symptoms, like hay fever, to life-threatening, systemic reactions like anaphylactic shock. This underlines the potential hazard of high systemic IgE titres. Remarkably, over the last four decades the incidence of allergic disease has risen. This represents an intriguing problem from a medical, epidemiological, immunological, genetic and evolutionary view. Unfortunately, it is also a major socio-economic problem. One interpretation of these data is that control mechanisms, which were adequate in the past and honed in evolution, are failing.

In the recent past others and we have described several B-cell-specific control mechanisms that indicate a tight control of the IgE response, in agreement with the arguments mentioned above, and that are different from the opsonic type of response (Fig. 2.1):

1. Reduced IL-4-dependent class switch recombination (CSR) to the ε heavy chain (ε-HC) locus in comparison to the γ-1 (γ1)-HC locus [5].
2. Short half-life of free IgE in serum, limiting the risk of a systemic anaphylactic reaction [6].
3. Negative feedback function of CD23, the “low” affinity receptor for IgE, resulting in an in-time and quantity-restricted response [7].
4. Direct impact of the membrane (m)IgE receptor on the quality and quantity of the IgE response in vivo [8, 9].
5. Poor expression of mRNA for the membrane form of both the murine and the human ε-HC, but not for the murine γ1- and the corresponding human γ4-HC [8, 10], resulting in limited expression of IgE as a membrane-bound, antigen-receptor-type molecule [8, 9, 11].
6. Lower chance to contribute to the long-lived plasma cell pool and thus to humoral immunologic memory [12].

In the present review we want to describe these molecular mechanisms and discuss their biological impact on the IgE level in detail.

2.2 Reduced Class Switch Frequency to the IgE Locus

During an immune response, B lymphocytes can switch the Ig isotype from IgM to IgG, IgE, or IgA. This Ig-CSR is based on a DNA recombination event that results in an exchange of the gene segments coding for the constant region of the Ig heavy chain, while retaining the Ig heavy chain variable region. This process changes the effector functions of the corresponding antibody. Much of our current

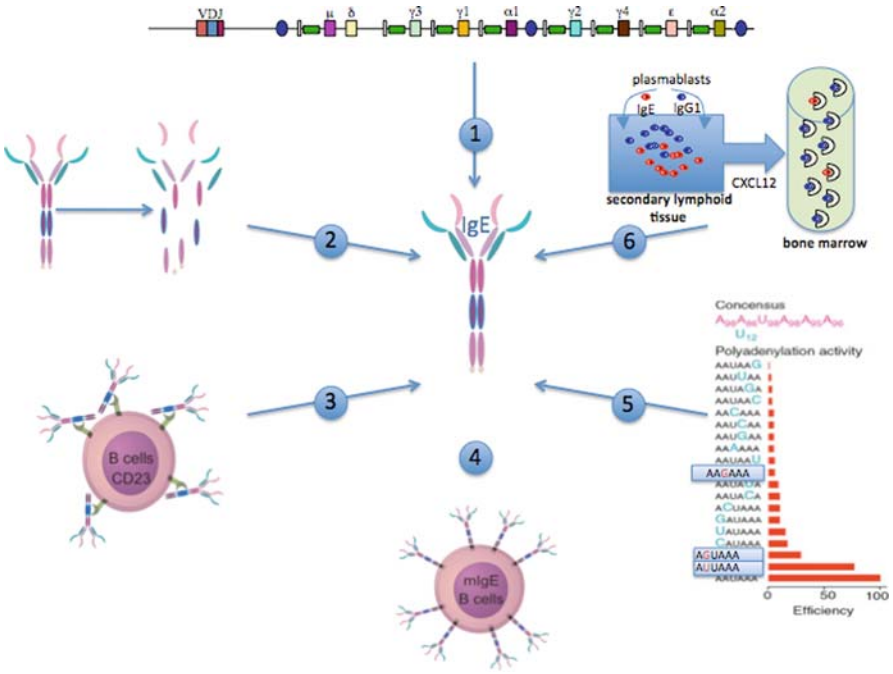

Fig. 2.1 Schematic overview of B-cell-specific control mechanisms, tightly regulating IgE expression in vivo (1) class switch recombination: Although class switching to IgE and IgG1 is induced by the same cytokine milieu followed by similar signal transduction pathways, a 6-fold reduced switching frequency to IgE is observed. (2) Serum half-life: IgE was reported to degrade between 5 and 12 hours, thus displaying the shortest half-life of all immunoglobulin isotypes. (3) Negative feedback regulation by CD23: CD23-knockout mice show a 6-fold increase in serum IgE level. (4) The IgE antigen receptor: Regulation of quantity and quality of the IgE response directly correlates with the surface expression of mIgE. (5) Alternative polyadenylation: In contrast to all other isotypes, the mIgE-RNA gets polyadenylated by three cryptic poly(A) sites. (6) Plasmablast migration: IgE plasmablasts have an intrinsic lower chance to contribute to humoral memory than IgG1 plasmablasts

understanding of the molecular mechanisms of CSR is based on the analysis of in vitro switched B cells where murine and human naïve B cells can be activated by bacterial lipopolysaccharides (LPS), anti-CD40, or CD40L to undergo CSR. Cytokine signals can direct CSR to distinct classes, e.g. interleukin-4 (IL-4) will target murine IgG1 and IgE, and human IgG4 and IgE, respectively. Class switch to IgE is a very rare process [5], which is tightly regulated and can thus be considered as a further means to keep serum IgE low.

CSR (Fig. 2.2) requires transcription of the S regions, which are located upstream of each isotype constant exon and which subsequently become a substrate for the activation-induced deaminase (AID). AID was first discovered by Muramatsu et al. [13] and soon turned out to be the central enzyme in CSR and hypermutation [14]. AID belongs to the RNA editing deaminase family. It could be shown that AID actually acts on DNA of Ig VDJ and S regions rather than on RNA, thereby deaminating

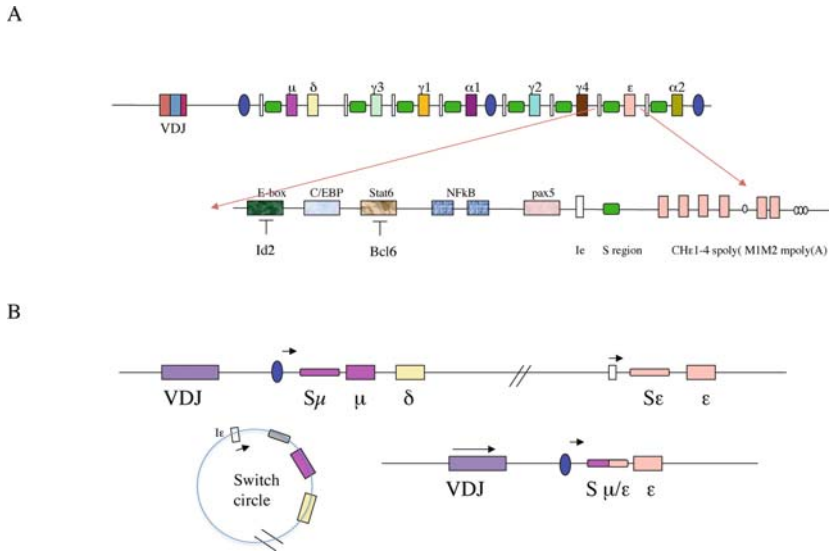

Fig. 2.2 The human immunoglobulin locus exhibits an upstream VDJ arrangement followed by several constant regions of different isotypes (**A**). Each isotype (except IgD) consists of a switch region, which has an upstream promoter/I exon region and downstream constant exons for the secreted Ig molecule followed by a poly(A) site for secreted antibody followed by exons M1 and M2 coding for transmembrane and cytoplasmic domains of the respective isotype and a poly(A) site for the membrane version. Also indicated are the promoters of the V, D and I exons and the internal enhancer elements as well as the 3′ enhancer. Identified transcription factor sites for the Iε promoter are indicated. (**B**) Upon transcription of the S region, RNA and DNA form a stable R-loop and AID gets access to the S regions and deaminates them causing U/G lesions. These lesions are causing DNA breaks, which ultimately lead to DNA recombination and excision of the intervening DNA as a switch circle

C to U [15]. The generated uracils are subjected to general repair mechanisms, which cause single- and double-stranded DNA breaks [16]. Joining of these ends, presumably by non-homologous end joining mechanisms, ultimately leads to CSR and the excision of the intervening DNA in a switch circle [17]. The central role for AID in CSR and hypermutation was not only shown in AID knockout mice [14] but also in patients who harbour defective AID gene expression or non-functional AID mutants [18]. Further elements are thought to contribute to the Ig isotype-specific targeting of CSR, such as the exact composition of the S region—including stem loop structures, chromatin and DNA modifications, promoter regions and germline transcription (GLT) levels (*cis*-acting elements) – as well as *trans*-acting factors that target the CSR machinery to distinct S regions.

Evidence for the necessity of GLT preceding CSR has come from studies in which deletion of promoter elements that abolished GLT also impaired CSR [19]. Vice versa, enforced transcription of S regions by constitutive or inducible promoter elements leads to induced CSR. This could be shown in knock-in experiments [20] as well as in artificial switch substrates introduced into B-cell lines and even in non-B cells that ectopically express transgenic AID [21]. In any S region, GLT initiates

from a promoter upstream of the germline CSR region (S region). The GLTs comprise a small 5′ exon—termed I exon, which is located upstream of the respective S region—spliced to the normal constant heavy chain exons with the intervening S region spliced out from the primary transcript [22]. To exploit GLT as a regulatory mechanism in isotype specificity of CSR, each germline promoter consists of a unique set of transcription factor (TF)-binding sites. In case of the ε germline promoter, it could be shown that treatment of primary B cells with the mitogen LPS and IL-4 is sufficient to trigger GLT from IgE [23].

Mitogenic signals like LPS or stimulation of CD40 [24] or signals transmitted by BAFF/April interacting with BAFFR/TACI/BCMA on B cells [25] lead to activation of NF-κB, which binds to the ε promoter together with the TF Stat6 that becomes activated upon IL-4/IL-13 signalling [26]. As shown in Fig. 2.2, several other TFs were identified, which can bind to the ε GL promoter like B-cell-specific activation protein (BSAP or Pax5) [27], c-Rel [28], C/EBP and AP1. AP1 only transactivates ε GLT from the mouse, but not from the human promoters [29]. TFs that repress transcription from the ε promoter are B-cell lymphoma 6 (Bcl-6) and Id2. Bcl-6 was shown to repress IL-4-induced CSR by competing for Stat6-binding sites within the ε and γ1 promoters [30] and Id2 binds to E2A TFs, thereby inhibiting their binding to ε promoter elements [31]. Possibly, TFs of the E2A group (E12 and E47) might also play a role in targeting AID to distinct promoter elements of the Ig locus [32] where AID might interact with the transcriptional complex to deaminate S regions [33]. In mouse cell lines, also the TF Ikaros was shown to dampen IgE and IgG GLT by binding to the germline promoter [34]. Schaffer et al. [35] showed that the homeodomain proteins HoxC4 and Oct-1 could bind to Iγ and Iε promoters to decrease levels of GLT and thus diminish CSR to IgG and IgE [35, 36]. There is also evidence for the 3′ enhancer of the Ig locus to selectively interact with promoter regions of the GLTs, in particular with the GL ε and γ2b promoters, which might help to target the recombination machinery to the distinct switch regions [37–39]. However, GLT without splicing of the primary transcript is not sufficient to target switch recombination as deletion of the splice donor site of the Iγ1 exon impaired CSR to IgG1.This shows that CSR also requires processing of the GLTs [40]. Stimulation of B cells with mitogenic signals and IL-4 not only induces GLT from the IgE—but also from IgG1−promoter, which exhibits similar TF-binding sites in its promoter region [41].

The observation that in single B cells more than one isotype-S regions are transcribed [42], together with the fact that AID-mediated DNA deamination is primarily restricted to the Ig locus and is not coupled to all genes that are transcribed in the B cell [22], implies that there have to be further levels of isotype-specific CSR regulation. Initially, it was proposed that due to the high GC contents, S regions are forming stable RNA–DNA hybrids (R-loops) upon transcription, in which the non-template strand remains single stranded and thus serves as a substrate for AID-mediated deamination [43]. However, AT-rich S regions, which are not prone to stable R-loop formation, are also effectively targeted by the CSR machinery, which implies the existence of R-loop-independent AID-recruiting mechanisms [44]. Larson et al. [45] propose that the tertiary G4 DNA structure of transcribed S regions allows specific attraction of repair proteins to S regions, thereby promoting

DNA synapsis and recombination. In addition, histone modifications such as phosphorylation, acetylation and methylation were found to occur in S regions accessible for CSR [33, 46, 47], which were not necessarily coupled to changes in the level of GLT [48]. Additionally, S region length was shown to affect the efficiency of CSR [49]. From these data, it is tempting to speculate that slight differences in S region length, composition and tertiary structures might generate isotype-specific protein-binding sites responsible for isotype-specific targeting of AID to individual S regions. In addition, the C-terminal domain of AID was found to be important for CSR but not for hypermutation, which leads to the assumption that CSR-specific factors might interact with the C-terminal domain of AID to either target AID to specific S regions or to mediate specific DNA synapsis and recombination [50]. Currently, the only published interaction partner for AID is the DNA-binding protein RPA (replication protein A) [51], which binds to AID upon phosphorylation by protein kinase A [52]. Binding of RPA to AID in vitro enhances the ability of AID to deaminate cytidines within transcribed double-stranded DNA and probably targets AID to the S region DNA. Further factors that are important in regulating CSR by yet obscure mechanisms, which do not exclude physical interaction with AID/CSR machinery, are the TF bach2 [53], E47 [32] and swap70, the latter could be shown to have a positive effect specifically on the IgE response in mice [54].

The puzzling phenotype of patients with Job′s or hyper IgE syndrome [55–57] (chronic eczematous dermatitis, recurrent skin and sinopulmonary tract infections, mucocutaneous candidiasis, coarse facies and a remarkably elevated serum IgE level) now seems to be solved and sheds additional light on the complexity of IgE immune response. Job′s syndrome is caused by mutations in the DNA-binding domain or the SH2 domain of the TF Stat3 [58, 59]. Whereas the infectious traits of Job′s syndrome can be explained by a deficiency in Th17-cell development [60, 61], which severely impairs immune responses to certain bacteria and fungi, the extremely high IgE levels are more diversely discussed. Stat3 is involved in many signalling pathways [62] and the most important TF in IL-21 signalling. In the mouse IL-21 or IL-21R deficiency has been correlated with low levels of serum IgG1 and high levels of IgE [63]. This may be due to an inhibition of CSR to IgE, because IL-21 induces ID2 expression [64]. Stat3, however, also transduces signals for the IL-6 family and the IL-10 family of cytokines and can induce several effector cells, like T and NK cells to produce IFN-γ [62]. All these factors can by themselves influence CSR to the IgE locus and influence the amount of IgE produced [62]. The influence of many ILs and cytokines on the level of expression of IgE clearly incorporates IgE in ongoing immune responses, yet without a clear hint to its core function.

2.3 Serum IgE Has the Shortest Half-Life of All Serum Immunoglobulins

The half-lives of several sets of murine monoclonal antibodies (mAbs) expressing the same V region in combination with all isotypes of serum Igs were determined by Vieira et al. [6] (Table 2.1). IgE was reported to degrade between 5 and 12 hours

Table 2.1 Serum half-lives of immunoglobulins in rodents and humans

Immunoglobulin isotype	Serum half-life ($t^1/_2$)		
	Mouse[a]	Rat	Human[c]
IgM	2 days	1 days[e]	5–10 days
IgG1	6–8 days	2 days[e], 9–10 days[f]	21–24 days
IgG2	–	–	21–24 days
IgG2a	6–9 days	4–5 days[e], 9–10 days[f]	–
IgG2b	4–6 days	2–3 days[f]	–
IgG2c	–	4 days[f]	–
IgG3	6–8 days	–	7–8 days
IgG4	–	–	21–24 days
IgA	17–22 hours	27 hours[e]	–
IgA1	–	–	5.9 days
IgA2	–	–	4.5 days
IgE	12 hours[a], 5–8 hours[b]	13.1 ± 5.7 hours[d]	1–5 days

[a]Vieira et al. [6], [b]Haba et al. [65], [c]Leffell et al. [168], [d]Hanashiro et al. [67], [e]Peppard and Orlans [169] and [f]Medesan et al. [170].

[6, 65], thus displaying the shortest half-life of all Ig isotypes. Additionally, alterations in the half-life of IgE were reported in dependency of the site of application. Hirano et al. [66] published that intravenously injected murine anti-DNP-IgE persisted for 12 hours whereas intradermally injected IgE was stable for at least 6 days. Similar half-lives for rat IgE (13.1 ± 5.7 hours) were published by Hanashiro et al. [67].

Waldmann et al. [68] hypothesized that an increased catabolic rate of IgE is dependent on the existence of intravascular and/or extravascular compartments. Human IgE is metabolized mainly in the extravascular compartment and the catabolism of IgE is related to the interaction of IgE with Fcε-receptor (FcεR)-bearing cells. In contrast, it has also been speculated that the vascular endothelium represents a site of catabolism of IgE. Interestingly, an FcRn (Brambell receptor [69]) knockout reduced the serum half-life of IgG1 in mice from 9 to 1.4 days. Thereby, IgG1 in FcRn-knockout mice has roughly the same half-life as all other Ig isotypes in mice [70]. Moreover, Lu et al. [71] generated transgenic mice that overexpressed the bovine FcRn (bFcRn) in their lactating mammary glands. Significantly increased IgG levels were observed in the sera and milk from transgenic animals, suggesting that the overexpressed bFcRn binds and protects endogenous mouse IgG and thus extends its life span. These results indicate that the main reason for the difference in the half-life time between IgG and IgE is explained by the stabilizing interaction between IgG and FcRn. Additionally, the MHC class I-related protein FcRn, originally identified by Simister and colleagues [72, 73] plays a critical role in IgG homeostasis by protecting IgG from normal protein catabolism, which results in a substantial increase in the half-life of IgG.

Similarly, the interaction between IgE and FcεRI stabilizes both partners, increasing the half-life of cell-bound IgE to months. The decreased sensitivity to decay

is accompanied by a conformational change in the three-dimensional structure of IgE: binding to the FcεRI causes the protein to open up from a bent shape into a more stretched shape, leaving the antigen-binding sites intact [4]. The majority of IgE in the body is probably bound to FcεRI on basophils and mast cells, whereas IgG is primarily found in the free, circulating form. Nevertheless, the short half-life of unbound (free) IgE in the blood limits the danger of a systemic anaphylactic reactions.

2.4 CD23 Influences IgE Expression by a Negative Feedback Inhibition

In humans two isoforms, CD23a and CD23b, differing in the first six cytoplasmic amino acids [74, 75] were described. While CD23a is constitutively expressed on B cells and follicular dendritic cells, CD23b, after IL-4 stimulation, is expressed on a variety of hematopoietic cell types like B cells, monocytes, eosinophils and Langerhans cells [75–77]. For a long time only one CD23 isoform was known in mouse, corresponding to human CD23a, but recent studies also described the existence of the CD23b isoform. However, CD23 expression in mouse is restricted to B cells (CD23a) and follicular dendritic cells (CD23a and CD23b) [78–80].

Unlike other Fc receptors, CD23 does not belong to the Ig superfamily of proteins, but is a 45 kDa type II transmembrane glycoprotein comprising a C-terminal lectin domain, followed by a stalk region, a transmembrane domain and a short cytoplasmic tail [81, 82]. The lectin domain binds IgE via the Cε3 domain in a calcium-dependent way [83, 84]. CD23 is expressed on the cell surface as trimers [82, 85]. Oligomerization mediates high-affinity binding to IgE, where two lectin heads bind to one IgE molecule [82]. CD23 binds IgE with both a medium affinity ($4–10 \times 10^7$ M^{-1}) and a low affinity ($4–10 \times 10^6$ M^{-1}) [86]. Besides this membrane-bound form (mCD23), CD23 also exists as soluble fragments of different sizes (sCD23) when cleaved by an autocatalytic mechanism mediated by the metalloprotease ADAM10 [87, 88]. With the exception of one soluble fragment, all of them contain the IgE-binding lectin domain and can bind IgE with low affinity in the range from 10^5 to 10^6 M^{-1} [89].

CD23 is a pluripotent molecule; its biological functions include cell activation and proliferation, cell adhesion, IgE-dependent antigen transport, processing and presentation as well as regulation of IgE synthesis and expression. However, the most striking phenomenon is CD23′s ambiguous function on IgE regulation, both in activation and/or inhibition of IgE production. Several studies with CD23-deficient and CD23-overexpressing mice clearly demonstrated CD23′s role as a negative feedback regulator of IgE production [90–95]. Yu et al. [7] showed that disruption of the CD23 gene led to increased specific IgE levels after immunization with TD antigens. While IgG1 levels were twice as high after immunization with 2,4-dinitrophenyl-ovalbumin (DNP-OVA), specific IgE levels were 6–12 times higher in these mice. In contrast, mice overexpressing CD23 produce much lower amounts of IgE, confirming CD23 as a potent regulator of IgE production. Experiments with

New Zealand black mice, which cannot form trimers of CD23 and therefore show impaired high-affinity binding of IgE, also have an exaggerated IgE response [96]. Furthermore, anti-CD23 antibody treatment inhibits specific IgE responses and anti-stalk antibodies enhance IgE production and promote cleavage of mCD23 [97–102]. This increase can be attributed to the prevention of cooperative association of the IgE-binding lectin domains, therefore interfering with oligomerization and high-affinity binding of CD23 [85, 98]. Kilmon et al. [98] demonstrated that RAS1, an anti-stalk antibody, not only prevents oligomerization of CD23 but also inhibits the release of mCD23 [98]. Mice treated with RAS1 and immunized with KLH-DNP/Alum with pertussis toxin also expressed higher levels of IgE, comparable to $CD23^{-/-}$ mice. mAB 19G5 also inhibited high-affinity IgE binding and led to increased IgE levels, but unlike RAS1, 19G5 favoured cleavage of mCD23, leading to elevated sCD23 levels (100–150-fold) [85, 103]. Kinetics of the IgE response were comparable with a primary immune response. Thus, here the elevated serum levels were simply the result of cytophilic release of CD23-bound IgE. Ford et al. [103] also showed that IgE levels are increased even in the absence of Ag-alum, indicating that CD23′s negative regulation of IgE can be abrogated by destabilizing CD23. These results clearly revealed CD23′s ambiguous function on IgE regulation: while stabilized mCD23 negatively regulates IgE production, destabilization of mCD23 leads to an increase of IgE.

Summarizing, in the early phase, IL-4 activates expression of both IgE and CD23, whereas later on, when IgE levels have reached a certain threshold, binding of IgE to CD23 stabilizes and prevents degradation of CD23. This inhibits ongoing IgE synthesis and dampens the immune reaction. However, CD23 seems to play only a role in regulating moderate amounts of IgE: first, helminth-infected wild-type and $CD23^{-/-}$ mice showed the same IgE response [7] and second, destabilization of mCD23 via 19G5 treatment did not lead to an increase of IgE in helminth-infected mice [103].

Many studies have been performed to address the mechanisms behind CD23′s regulatory function. Aubry et al. [104] showed that sCD23 enhances IgE synthesis by binding to CD21 on peripheral blood B cells. Christie et al. [105] prevented autocatalytic cleavage of mCD23 and measured reduced IgE levels. In contrast, Texido et al. [92] showed that transgenic mice for sCD23 exhibited no phenotype, indicating that it is the membrane-bound form of CD23 that regulates IgE production. Ford et al. [103] investigated the role of CD21, a natural ligand for CD23. By using CD21-deficient mice it could be demonstrated that the 19G5-induced IgE response is independent of CD21 signalling, suggesting that increased IgE levels can be attributed to the loss of mCD23 and the release of CD23-bound IgE and not to the accumulation of sCD23 [103]. Recent studies from McCloskey et al. [106] showed that sCD23 both inhibits and stimulates IgE production, depending on the structure of the fragments: while sCD23 monomers inhibit IgE synthesis in human B cells, oligomers stimulate synthesis by co-ligating IgE and CD21 on IgE^{+} B cells.

Summarizing, binding of IgE to mCD23 stabilizes the trimer and prevents its degradation, thus inhibiting the autocatalytic release of sCD23 fragments, which stimulate (in oligomeric form) IgE synthesis. Data from McCloskey et al. [106]

confirmed the competition model between CD23 and CD21 for membrane-bound IgE on the B-cell surface proposed by Hibbert et al. [107]. Co-cross-linkage of mCD23 and mIgE by an IgE/Ag complex results in a decrease of IgE; co-cross-linking of CD21 and mIgE by sCD23 leads to an increase of IgE [107]. Still, the mere absence of CD23, as we have seen, suffices to increase IgE responses.

2.5 The Biological Function of the mIgE Antigen Receptor on IgE Synthesis In Vivo

The B-cell receptor (BCR) is undoubtedly the most important component of a B cell′s interface regarding communication with the local environment. Main developmental steps taking place in the bone marrow like signalling through the pre-BCR as well as avoiding autoreactivity by clonal deletion or receptor editing utterly rely on the expression of a functional receptor. In the periphery, the BCR is one of the driving forces establishing antibody responses addressing invading pathogens and it has been shown to be essential for maintaining peripheral B-cell tolerance in the case of B-cell anergy [108]. Regarding mIgE, the relevance of the receptor seems restricted to responding secondary lymphoid organs like the spleen, lymph nodes and Peyer′s patches. Here, the decision to switch to $mIgE^+$ plasmablasts and finally to IgE-producing antibody-secreting cells (ASCs) is being made in an adequate cognate T-cell-help and cytokine context [109].

mIgE, in contrast to its soluble form, contains three additional structural features encoded by exon M1 and M2, namely the *EMPD* (extracellular membrane-proximal domain) domain, the *transmembrane domain*, which anchors the receptor in the cell membrane and serves as interaction domain for the CD79α/β sheath to form the BCR [110, 111] and the *cytoplasmic domain*. The EMPD regions of the five isotypes differ in length and amino acid composition. In human IgE two functional forms of ε_{EMPD}, namely ε_{short} and ε_{long}, exist composed of 14 and 66 amino acids, respectively [112–114]. According to a study of Poggianella et al. [115] carried out in the mature murine B cell line A20, the presence of the EMPD region is of critical importance for mobilizing intracellular Ca^{2+}, with the EMPD′s length apparently being the "sensor" of caspase-independent apoptosis sensitivity. A similar phenomenon of inhibition of proliferation has been reported in murine WEHI-231 cells transfected with the shorter human version of mIgE [116]. This study also showed that the rate of transport by which the two forms are brought to the cell surface as well as the association with CD79α and the kinetics of protein tyrosine phosphorylation in response to receptor cross-linking differs between the short and the long version. Thus, the form of the EMPD region might have an essential function in shaping the repertoire of $mIgE^+$ plasmablasts selected towards the long-lived plasma cell fate.

A step forward in the understanding of the role of mIgs other than mIgM or mIgD was achieved with two mouse lines with mutations in the ε-HC gene. In the first, the intracellular domain of IgE was removed except for three amino acids (Lys, Val and Lys) (KVKΔtail line). The cytoplasmic domain of IgE in these mice is the same as