Medical Hypotheses 79 (2012) 519–521

Contents lists available at SciVerse ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Confirmation of the "protein-traffic-hypothesis" and the "protein-localization-hypothesis" using the diabetes-mellitus-type-1-knock-in and transgenic-murine-models and the trepitope sequences

Borros Arneth *

Institute of Clinical Chemistry and Laboratory Medicine, Carl Gustav Carus University Hospital, Medical Faculty of the TU-Dresden, Fetscherstr 74, Dresden, Germany

ARTICLE INFO

Article history:
Received 11 November 2011
Accepted 8 July 2012

ABSTRACT

As possible mechanisms to explain the emergence of autoimmune diseases, the current author has suggested in earlier papers two new pathways: the "protein localization hypothesis" and the "protein traffic hypothesis". The "protein localization hypothesis" states that an autoimmune disease develops if a protein accumulates in a previously unoccupied compartment, that did not previously contain that protein. Similarly, the "protein traffic hypothesis" states that a sudden error within the transport of a certain protein leads to the emergence of an autoimmune disease.

The current article discusses the usefulness of the different commercially available transgenic murine models of diabetes mellitus type 1 to confirm the aforementioned hypotheses.

This discussion shows that several transgenic murine models of diabetes mellitus type 1 are in-line and confirm the aforementioned hypotheses. Furthermore, these hypotheses are additionally inline with the occurrence of several newly discovered protein sequences, the so-called trepitope sequences. These sequences modulate the immune response to certain proteins. The current study analyzed to what extent the hypotheses are supported by the occurrence of these new sequences.

Thereby the occurrence of the trepitope sequences provides additional evidence supporting the aforementioned hypotheses.

Both the "protein localization hypothesis" and the "protein traffic hypothesis" have the potential to lead to new causal therapy concepts.

The "protein localization hypothesis" and the "protein traffic hypothesis" provide conceptional explanations for the diabetes mouse models as well as for the newly discovered trepitope sequences.

Introduction/background

The existence of autoreactive T-cells in healthy mammalian organisms has been described extensively by Père et al. [1], by Donath and Shoelson [2], and by others and thus far accepted. These T-cells remain suppressed in a healthy organism by regulatory T-cells (Treg cells) that prevent their expansion. We discuss why and how autoreactive T-cells develop in healthy mammals and consider whether differences in the specificity of autoreactive T-cells mediate the emergence of autoimmune diseases, addressing the question of whether healthy persons and patients have disparities in the specificities of their autoreactive T-cells?

The hypotheses/ theory

To address the emergence of autoimmune diseases, the author of this article has described two hypotheses in earlier papers:

(1) The first hypothesis is the "protein localization hypothesis". This hypothesis suggests that the localization of a protein is a crucial determinant of its antigenicity as described by Arneth [3].
(2) The second hypothesis is the "protein traffic hypothesis" and was proposed by the author in 2010 [4]. Building on the "protein localization hypothesis", the "protein traffic hypothesis" implies that changes in the localization of a protein due to a misguided signal in the protein transport system may promote the emergence of an autoimmune disease.

Evaluation of the hypotheses

The best experimental confirmation of one or both of the aforementioned hypotheses would be the identification of coding genes that carry a mutation in the signal sequence and/or the cloning of genes with a mutation or variation within the signal sequence and insertion of these genes at defined loci in model organisms. Thereafter, these mutated or cloned genes could be expressed in a mouse or other animal model. In principle, this type of expression is

* Tel.: +49 351 458 12612; fax: +49 351 458 2612.
E-mail address: borros.arneth@uniklinikum-dresden.de

0306-9877/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.mehy.2012.07.009

achieved via naturally occurring mutations or polymorphisms and can be achieved experimentally using 'established knock-in mouse models and/or transgenic mice models, including those of diabetes type 1.

Several mutations and polymorphisms that are relevant to the insulin gene and the development of type 1 diabetes mellitus have been described by Masahiro and Kishio [5] and by Fendler et al. [6].

Furthermore, textbooks on molecular cloning have noted that the cloning of genes into mammalian cells must be performed using the DNA sequence of the entire gene, including the promoter region and especially the signal sequence, examples are the textbooks by Sambrook et al. [7] and Glover [8].

Empirical data I: the different commercially available insulin murine models

Transgenic murine models of diabetes mellitus type 1 are available and are well characterized. All of these murine models are commercially available from the Jackson Laboratory (The Jackson Laboratory, Bar Harbor, Maine, USA) and are described below.

(1) The insulin promoter is used and is located upstream of the inserted insulin-GFP gene sequences. The expressed protein is a fusion protein comprising insulin and green fluorescent protein (GFP) This transgenic mouse model has been described by Hara et al. [9–11], and by Katz et al. [12].
(2) The insulin promoter is used and is located upstream of the inserted gene sequences. The expressed protein is a fusion protein comprising human transferrin and ovalbumin. This transgenic mouse model has been generated and used by Morgan et al. [13] and by Kurts et al. [14].
(3) The rat insulin 2 promoter is used and is located upstream of the inserted gene sequences. The expressed protein is the chicken calmodulin protein. This murine model has been established by Epstein et al. [15].
(4) The ins 1 insulin promoter is used and is located upstream of the inserted gene sequences. The expressed protein is the human C-peptide GFP (hProCpepGFP) propeptide, which is a fusion protein comprising the human C-peptide and GFP. This transgenic mouse was first described by Hodish et al. [16].

All of these different transgenic murine models share several features:

(1) The expression of a novel protein sequence, which is typically a modified insulin peptide, is controlled using a fixed promoter such as the insulin promoter.
(2) Therefore, the protein expression of a novel protein sequence occurs at a later stage in development.
(3) Under the control of the insulin promoter, both proteins with unaltered sequences and proteins that have been genetically altered are expressed in these organisms. Furthermore, exogenous or endogenous stimuli (insulin) provoke the generation and expression of new protein sequences.
(4) The expression of new or foreign proteins leads to the emergence of diabetes mellitus type 1. For the export of a protein into the extracellular space, the presence of an export signal sequence (encoding the signal peptide) is crucial.
(5) This raises the important considerations, whether a signal sequence is cloned together with the protein sequence, whether an export sequence is also cloned, and whether this modified new export sequence is efficient. Without insertion of an export sequence, the resulting protein remains intracellularly localized. Therefore, a new peptide is expressed and subsequently accumulates in the intracellular space as a function of the insulin promoter.

If the accumulation of this protein results in a critical intracellular concentration, this peptide will be recognized as foreign and should cause subsequent activation of the immune system. Therefore, this activity may mediate the development of diabetes mellitus type 1 via islet cell inflammation (insulitis), which has been observed in transgenic murine models. Transgenic murine models can therefore be used to confirm the protein localization and protein traffic hypotheses. Furthermore, diabetes mellitus type 1 can be used as a model for the emergence of other autoimmune diseases. Several other autoimmune diseases may develop in a manner similar to that described in this article due to dysfunctional gene activation, or due to mutations in promoters that cause an over-expression of one or more genes. In addition, errors or mutations in the signal sequences may misguide protein trafficking and perturb protein localization. In this regard, transgenic murine models including that of diabetes mellitus type 1 might exhibit general characteristics that are applicable to the emergence of at least several autoimmune diseases.

The mechanism that is described in this article may reflect a general mechanism. The murine models that are described in this article might serve as model organisms for the demonstration of fundamental principles. Transgenic mice are used to model autoimmune diseases. Based on the exact location of the promoter, the mRNA may include an export sequence. The presence or absence of a signal peptide typically depends on the type of protein that triggers the development of certain autoimmune diseases. Transgenic tissue, which should express the new protein, may be attacked by the immune system if the level of protein expression perturbs protein localization. Autoimmune disease may occur if a foreign protein is suddenly expressed or is suddenly expressed in a higher amount. In complementary transgenic murine models, the mice should not develop an autoimmune disease if native murine extracellular proteins are over-expressed because the new gene product contains an export sequence. Heyser et al. [17] have developed the IL6 transgenic mouse, which is an example of this scenario.

Diabetes mellitus type 1 is also induced in several murine models following immunization with insulin and/or with insulin DNA as described by Pechhold et al. [18], by Karges et al. [19], and by Paronen et al. [20], but also by others.

Empirical data II: the occurrence of trepitope sequences

The theories that are proposed above are consistent with the recent new discovery of the T cell regulatory epitopes, which are called trepitopes. Trepitopes are protein sequences that determine the antigenicity of a given protein and its behavior to regulatory T cells. Six trepitope sequences have been described for IgG, and two sequences have been described for the albumin molecule as described by De Groot et al. [21]. The analysis of these sequences indicates that all of the sequences that have been previously described are for extracellular body-own proteins. This analysis is consistent with the separation of the T cell receptor spectrum and the T cell receptor repertoire and with the protein localization and protein traffic hypotheses that are described in this article. If various proteins have distinct destinations, these proteins must be differentially recognized by the immune system. The differences in recognition are indicated by different sequences in the protein structure. These different sequences of various proteins (intracellular versus extracellular proteins), which can be evaluated by performing a search of the human genome using the NIH Blast algorithm, also support the aforementioned hypotheses. Therefore, the presence of trepitope sequences is consistent with the aforementioned hypotheses. The relevant trepitope sequences are exclusively found in extracellular and/or serum proteins, such as

IgG and albumin. The IgG trepitope sequences are PAVLQSSGLYSLSSVVTVPSSSLGTQ, which is the (EPX-167) sequence, E EQYNSTYRVVSVLTVLHQDW, which is the (EPX-289) sequence, and KVQWKVDNALQSGNSQ, which is the (EPX-134) sequence. The trepitope sequences on albumin are HPDYSVYLLLRLAKT and LLLRLAKTYETTLE, which are the albumin (EPX-362–376) and the albumin (EPX 369–382) sequences, respectively as described by De Groot et al. [21].

Consequences of the hypothesis and discussion

Both the protein localization and the protein traffic hypotheses as presented are in-line with both the different murine models and the occurrence of trepitope sequences. The murine models demonstrate what happens if a protein is expressed in the cells of an organism with and without signal peptides. In addition, the existence of several trepitope sequences that are selectively expressed in extracellular proteins suggests that extracellular proteins are somehow fundamentally different from intracellular proteins.

So both the transgenic murine models and the occurrence of the trepitope sequences on extracellular and serum proteins only are in agreement with the previously described hypotheses. To experimentally verify the initially described hypotheses, further more murine models with and without a signal sequence could additionally be generated by cloning genes into mice. Previous studies have evaluated the described transgenic mice models and provide evidence supporting the aforementioned hypotheses. The occurrence of the trepitope sequences is also consistent with these hypotheses. However, trepitope sequences cannot serve as a proof alone. Together with the murine models, the identification of the trepitope sequences supports the hypotheses that extracellular and serum proteins are recognized via immune mechanisms different from those that recognize intracellular proteins. The protein localization and protein traffic hypotheses represent two novel mechanisms completing each other regarding the genesis of autoimmune diseases. The wide range of possible misdirected proteins in each cell might fit with the diverse aspects of autoimmune diseases. Thereby the multiplicity of possible misdirected proteins in each cell and the diversity of aspects of the different autoimmune diseases might be linked to each other. Therefore, autoimmune diseases can be recognized as a class of diseases with characteristics distinct from those of other diseases. The class of autoimmune diseases includes so different diseases as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus. Thereby each of these diseases does not represent one uniform disease but are instead classes of different entities.

Conflict of interest

None declared.

References

[1] Père H, Montier Y, Bayry J, Quintin-Colonna F, Merillon N, Dransart E, et al. A CCR4 antagonist combined with vaccines induces antigen-specific CD8+T cells and tumor immunity against self antigens. Blood 2011;118(18):4853–62.
[2] Donath M, Shoelson S. Type 2 diabetes as an inflammatory disease. Nat Rev Immunol 2011;11:98–107.
[3] Arneth B. A new paradigm in biology – the effect of localization of a protein on its antigenicity. Iran J Immunol 2008;5(4):226–8.
[4] Arneth B. Experimental confirmation of the 'protein traffic hypothesis' by routine diagnostic tests. Int J Immunol Studies 2010;1(2):163–8.
[5] Masahiro N, Kishio N. Insulin gene mutations and diabetes. J Diabetes Invest 2011;2(2):92–100.
[6] Fendler W, Borowiec M, Antosik K, Jaroszewska-Swiatek B, Szwalkiewicz-Warowicka E, Malecki M, et al. Paternally inherited proinsulin mutations may result in earlier onset of monogenic diabetes mutation identity effect in monogenic diabetes. Diabetes Care 2011;34(1):e9.
[7] Sambrook, Fritsch, Maniatis, Molecular cloning a laboratory manual. Chapter expression of cloned genes in cultured mammalian cells, 2nd ed., 2002, p. 13.3.
[8] Glover. DNA cloning a practical approach, vol. III. Oxford: IRL Press; 2002.
[9] Hara M, Wang X, Kawamura T, Bindokas V, Dizon R, Alcoser S, et al. Transgenic mice with green fluorescent protein-labeled pancreatic cells. Am J Physiol Endocrinol Metab 2003;284:177–83.
[10] Hara M, Wang X, Kawamura T, Bindokas VP, Dizon RF, Alcoser SY, et al. Transgenic mice with green fluorescent protein-labeled pancreatic beta-cells. Am J Physiol Endocrinol Metab 2003;284(1):177–83.
[11] Hara M, Dizon RF, Glick BS, Lee CS, Kaestner KH, Piston DW, et al. Imaging pancreatic beta-cells in the intact pancreas. Am J Physiol Endocrinol Metab 2006;290(5):1041–7.
[12] Katz JD, Wang B, Haskins K, Benoist C, Mathis D. Following a diabetogenic T cell from genesis through pathogenesis. Cell 1993;74(6):1089–100.
[13] Morgan DJ, Kurts C, Kreuwel HT, Holst KL, Heath WR, Sherman LA. Ontogeny of T cell tolerance to peripherally expressed antigens. Proc Natl Acad Sci USA 1999;96(7):3854–8.
[14] Kurts C, Heath WR, Carbone FR, Allison J, Miller JF, Kosaka H. Constitutive class I-restricted exogenous presentation of self antigens in vivo. J Exp Med 1996;184(3):923–30.
[15] Epstein PN, Overbeek PA, Means AR. Calmodulin-induced early-onset diabetes in transgenic mice. Cell 1989;58(6):1067–73.
[16] Hodish I, Liu M, Rajpal G, Larkin D, Holz RW, Adams A, et al. Misfolded proinsulin affects bystander proinsulin in neonatal diabetes. J Biol Chem 2010;285(1):685–94.
[17] Heyser CJ, Masliah E, Samimi A, Campbell IL, Gold LH. Progressive decline in avoidance learning paralleled by inflammatory neurodegeneration in transgenic mice expressing interleukin 6 in the brain. Proc Natl Acad Sci USA 1997;94(4):1500–5.
[18] Pechhold K, Chakrabarty S, Harlan DM. Cytotoxic T cell-mediated diabetes in RIP-CD80 transgenic mice: autoantigen peptide sensitivity and fine specificity. Ann N Y Acad Sci 2007;1103:132–42.
[19] Karges W, Pechhold K, Al Dahouk S, Riegger I, Rief M, Wissmann A, et al. Induction of autoimmune diabetes through insulin (but not GAD65) DNA vaccination in nonobese diabetic and in RIP-B71 mice. Diabetes 2002;51(11):3237–44.
[20] Paronen J, Liu E, Moriyama H, Devendra D, Ide A, Taylor R, et al. Genetic differentiation of poly I:C from B:9-23 peptide induced experimental autoimmune diabetes. J Autoimmun 2004;22(4):307–13.
[21] De Groot AS, Moise L, McMurry JA, Wambre E, Van Overtvelt L, Moingeon P, et al. Activation of natural regulatory T cells by IgG Fc–derived peptide "Tregitopes". Blood 2008;112:3303–11.