Inhibition of diabetes in BB rats by virus infection.

T Dyrberg, … , P L Schwimmbeck, M B Oldstone

J Clin Invest. 1988;81(3):928-931. https://doi.org/10.1172/JCI113405.

Research Article

BB rats serve as a model for human insulin-dependent diabetes mellitus (IDDM), since without insulin treatment, most 60-140-d-old animals die within 1 to 2 wk of developing polyuria, polydypsia, hyperglycemia, and hypoinsulinemia. Lymphoid cells accumulate in the islets of Langerhans and beta cells undergo destruction. We report that inoculation of such BB rats with lymphocytic choriomeningitis virus (Armstrong strain, clone 13) reduces over a prolonged period the incidence of IDDM, normalizes the concentration of blood sugar and pancreatic insulin, prevents the mononuclear cell infiltration in the islets of Langerhans, and for a short time after inoculation alters T lymphocyte subsets. Thus, a virus might be programmed to carry out useful functions.

Inhibition of Diabetes in BB Rats by Virus Infection

Thomas Dyrberg, Peter L. Schwimmbeck, and Michael B. A. Oldstone
Research Institute of Scripps Clinic, La Jolla, California 92037

Abstract

BB rats serve as a model for human insulin-dependent diabetes mellitus (IDDM), since without insulin treatment, most 60–140-d-old animals die within 1 to 2 wk of developing polyuria, polydypsia, hyperglycemia, and hypoinsulinemia. Lymphoid cells accumulate in the islets of Langerhans and beta cells undergo destruction. We report that inoculation of such BB rats with lymphocytic choriomeningitis virus (Armstrong strain, clone 13) reduces over a prolonged period the incidence of IDDM, normalizes the concentration of blood sugar and pancreatic insulin, prevents the mononuclear cell infiltration in the islets of Langerhans, and for a short time after inoculation alters T lymphocyte subsets. Thus, a virus might be programmed to carry out useful functions.

Introduction

The BB strain of rats originating from the Bio Breeding Laboratory, Ottawa, Canada, serves as a model for the study of human insulin-dependent diabetes mellitus (IDDM)[1] (1, 2). The disease these rats develop manifests clinically as weight loss, polyuria, and polydypsia and chemically as hyperglycemia, glycosuria, and hypoinsulinemia (3). After the onset of IDDM with severe hyperglycemia, ketoacidosis develops rapidly; without insulin treatment, the rats usually die within a week. In the islets of Langerhans massive local accumulation of mononuclear cells is associated with a decrease in the number of beta cells and disruption of islet architecture (4). Although the etiology of this IDDM is not known, most likely the autoimmune response of BB rats to their own islet cells is an important pathogenic factor.

Previous studies from our laboratory have shown that infection of adult immunocompetent mice with a lymphotropic variant of lymphocytic choriomeningitis virus (LCMV) (Armstrong strain CA1371 53b, called clone 13) establishes persistent virus infection by specifically immunosuppressing the generation of LCMV specific H-2 restricted cytotoxic T lymphocytes (5). Clone 13 infects a small subset (1–2%) of circulating lymphocytes and causes a selective immunosuppression. Additionally, we and others have shown that several RNA and DNA viruses can infect monocytes and lymphocytes, causing alterations in a variety of immunologic responses (reviewed in reference 6). Depending on the time of inoculation and the host and virus used, immune responses can be either exaggerated to cause autoimmune responses or they can be suppressed.

The purpose of this study was to determine whether infection with LCMV, a relatively noncytopathic RNA virus, significantly reduced the expected incidence and chemical and histopathologic manifestations of IDDM in BB rats.

Methods

BB rats representing both diabetes-prone and resistant sublines in about the 20th generation of inbreeding (7) were obtained from the Worcester colony (Dennis Guberski, University of Massachusetts, Worcester, MA) and were propagated in our laboratory by brother–sister matings. IDDM was diagnosed as blood glucose levels of 300 mg/dl or more as measured by the stix-reflectometer method. Rats were weaned 30 d after birth and monitored for the occurrence of IDDM from the 45th d on, three times a week until they reached 240 d of age. Rats demonstrating IDDM, which developed at an average age of 103 d (range, 65–200) were either killed or treated with insulin (porcine protamine zinc insulin; Nordisk Gentofte, Gentofte, Denmark).

LCMV infection was induced by inoculating 30-d-old BB rats intravenously with 10^7 plaque-forming units (PFU) of LCMV clone 13. Details of virus history, passage, inoculation procedures, and plaque assay for LCMV infectivity have been described (5). Infectious center assay on PBMN and lymphocytes were performed as described (8). Purified subsets of PBLs were obtained by using specific MAbs to rat lymphocyte subsets (W3/13, pan T cell; W3/25, helper-inducer T cell; OX 8, nonhelper, cytotoxic, suppressor T cell, and natural killer cell), FITC fluorochrome dye, and FACS (9). Briefly, 1×10^6 lymphocytes were incubated with 50λ of MAbs at 4°C for 25 min. Cells were washed with media and stained with 5 μg affinity purified $F(ab)_2$ fragment anti-mouse IgG, washed, fixed in paraformaldehyde (1%), and quantitated by FACS. To detect LCMV RNA, tissues were harvested and RNA was extracted (10), denatured, and bound to nitrocellulose paper as described (10–12). The filters were hybridized with a 700-bp ^{32}P-labeled cDNA probe specific for the small RNA segment of LCMV-Armstrong (11).

The insulin concentrations in pancreatic tissues were measured as described (13). Briefly, pancreases were harvested, lyophilized, and dry weight measured. Tissues were homogenized and the insulin was extracted in acid ethanol. Insulin was quantitated using a competitive inhibition radioimmune assay with ^{125}I-A-14 porcine insulin as a tracer and rat insulin as the standard (13). For light microscopy, tissues were removed, fixed in Bouin's solution, and stained with hematoxylin and eosin. Chi-square test was used for statistical analysis.

Dr. Dyrberg's present address is Hagedorn Research Laboratory, 6 Niels Steensensvej, DK-2820 Gentofte, Denmark.

Received for publication 10 September 1987 and in revised form 16 November 1987.

1. *Abbreviations used in this paper:* IDDM, insulin-dependent diabetes mellitus; LCMV, lymphocytic choriomeningitis virus; LDV, lactate dehydrogenase virus; PFU, plaque-forming units.

J. Clin. Invest.

0021-9738/88/03/0928/04 $2.00
Volume 81, March 1988, 928–931

Results

As shown in Table I, 81% of the diabetes-prone BB rats spontaneously developed IDDM, whereas none of the diabetes-resistant BB rats did so. However, upon LCMV infection, 31 of 49 diabetes-prone BB rats (63%) failed to develop IDDM. The findings were highly significant (P value < 0.001) and were reproduced in three separate experiments. In support of these findings, analysis of pancreatic insulin levels revealed that the diabetic uninfected BB rats had little to no pancreatic insulin (mean±1 SE, 15±3 ng insulin per mg pancreas dry weight). In contrast, the insulin levels in the pancreases of diabetes-prone but LCMV-infected and non-IDDM BB rats were 13- to 15-fold higher (203±49). These levels were equivalent to those occurring in diabetes-resistant BB rats both uninfected and infected with LCMV (Table I). 37% or 18 of 49 diabetes-prone BB rats inoculated with LCMV did develop IDDM. These rats had blood glucose and pancreatic insulin levels similar to those in the uninfected, diabetes-prone BB rats that developed IDDM (Table I). Replicating virus was required as ultraviolet inactivation (360×10^3 rad/cm^2 treatment that inhibited all LCMV replication in vero cells: PFU $1 \times 10^8 \rightarrow < 50$) did not change the expected incidence of diabetes in diabetes-prone BB rats).

LCMV clone 13 is lymphotropic in mice and specifically suppresses their cytotoxic T lymphocyte response to the inoculating virus (5). Therefore, we looked for LCMV in lymphocytes of infected BB rats. Infectious virus was recovered from their lymphocytes 4, 7, and 10 d after inoculation but not after 30 d or longer. Titers ranged from 10^2 to 10^4 infective centers per 10^7 cells as demonstrated by cocultivation on Vero feeder cell layers. The sera of these animals contained infectious virus (10^2–10^5 PFU/ml) during the first 2 wk after viral inoculation and did not thereafter. RNA extracted from lymphocytes, spleens, livers, pancreases, adrenals, kidneys, and brains of LCMV-infected BB rats 4, 10, 60, and 170 d after initiating infection, when immobilized on nitrocellulose filters and probed with ^{32}P LCMV cDNA probe, failed to yield evidence of viral transcripts throughout the course of infection. Thus, with the combined use of these sensitive assays, LCMV was detectable only in lymphocytes using cocultivation, a more sensitive assay. All the LCMV-inoculated rats developed neutralizing antibodies to the virus (not shown).

Table I. LCMV Infection Significantly Reduced the Incidence of Diabetes

BB rats*	Diabetes‡	Number§	%	ng insulin per mg pancreas‖	Blood glucose¶
					mg %
Diabetes-prone					
Uninfected	+	94/116	81	15±3	>300
Uninfected	Nil	22/116	19	217±22	152±59
LCMV infect	+	18/49	37	17±3	>300
LCMV infect	Nil	31/49	63	203±49	163±5
Diabetes-resistant					
Uninfected	Nil	0/46	0	289±44	129±3
LCMV infect	Nil	0/18	0	227±69	131±8

* 30-d-old diabetes-prone BB rats were inoculated intravenously with LCMV-Armstrong clone 13, 10^7 PFU. Reduced incidence of diabetes was significant by chi-square test, $\chi^2 = 31.0$, $P < 0.001$.

‡ Diabetes (+) was defined as blood glucose level over 300 mg %. Once elevated, blood glucose levels remained high on repeated determinations unless rats were given insulin. Animals tested for insulin content of the pancreas were not given insulin for at least the four preceding days.

§ Number of rats with or without diabetes over total number per group.

‖ Pancreatic insulin levels were determined by a competitive radioimmune assay using ^{125}I-A-14 porcine insulin as a tracer and rat insulin as a standard. Mean±1 SE.

¶ Fasting blood glucose levels. Mean±1 SE.

Next lymphocyte subsets in virus-infected BB rats were studied. In accordance with an earlier report (1) diabetes-prone animals had a decrease in the number of T lymphocytes when compared with diabetes-resistant BB rats. As shown in Fig. 1, the number of T lymphocytes expressing a variety of T cell surface markers decreased significantly 4 and 7 d after viral inoculation of diabetes-prone BB rats when compared with uninfected controls of similar age and sex. The number of T lymphocytes in LCMV-infected rats returned to control levels by 14 d after inoculation and remained at normal levels thereafter. In contrast, analysis of T lymphocyte subsets of diabetes-resistant BB rats did not show marked changes. In addition, FACS-separated lymphocytes washed extensively and bearing W3/13$^+$, W3/25$^+$, or OX8$^+$ subsets yielded LCMV by cocultivation assay when tested 4 d after initiating the infection. Titers ranged from 1.2×10^3 to 2.4×10^3 infectious center per 10^7 sorted lymphocytes. In contrast, erythrocytes harvested from the same animal and washed in parallel failed to yield virus. These results further established that lymphocytes contain virus during the early stage of infection.

BB rats developing IDDM underwent massive infiltrations of mononuclear cells into the islets of Langerhans with a decrease in the number of beta cells and with severely disrupted islet architecture (Fig. 2). In contrast, rats infected with LCMV failed to develop either IDDM, inflammation in the islets of Langerhans, or damaged beta cells (Fig. 2). Histopathologically, the pancreases from LCMV-infected, diabetes-prone BB rats resembled those of their diabetes-resistant counterparts.

Discussion

Our observations indicate that soon after its introduction, LCMV somehow disorders particular lymphocyte subsets, resulting in reduced incidence of IDDM, inhibition of mononuclear cell infiltration, and destruction of islets of Langerhans. Lymphocytes from diabetic BB rats can transfer IDDM into resistant rats (14) and BB rats. IDDM can be inhibited by procedures directly interfering with lymphocyte functions or numbers, e.g., neonatal thymectomy (15) and treatment with lymphocyte antiserum (16) or cyclosporin A (17). LCMV caused a depletion of T lymphocytes at 4 and 7 d after initiating infection. Thus, the possibility exists that LCMV directly aborts autoimmune-producing lymphocytes. By this means, the virus could cause a long lasting immunosuppression equivalent to that observed with cyclosporin A or antilymphocyte serum. The recent cloning and sequencing of the clone 13 lymphotropic variant and the use of nucleic acid probes and in situ hybridization in association with the ability to incite IDDM by adoptive lymphocyte transfers should provide a unique opportunity to separate and identify the autoimmune

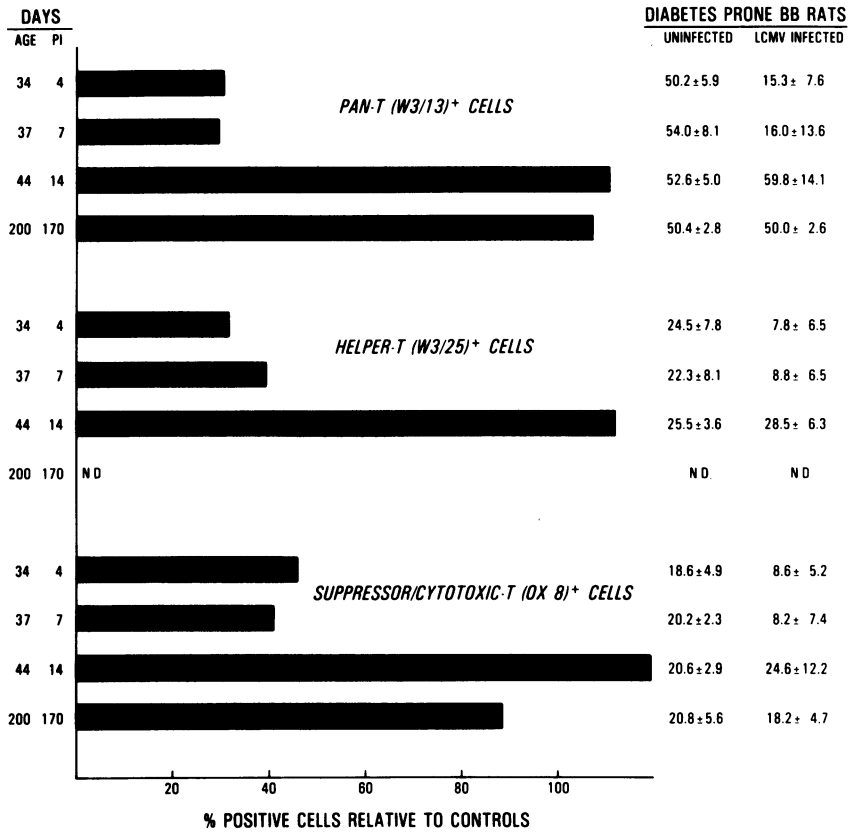

Figure 1. T lymphocyte subsets in diabetes-prone, LCMV-infected BB rats. Mononuclear cells, obtained from heparinized whole blood by gradient centrifugation on Ficoll-Hypaque, were incubated with the respective MAbs, washed, further incubated with FITC-F(ab_2) anti-mouse IgG, washed again, and fixed in paraformaldehyde. Cell-bound fluorescence was analyzed in the FACS. The proportion of W3/13, W3/25, and OX8 positive cells in LCMV-infected, diabetes-prone BB rats are shown relative to those in age-matched, uninfected, diabetes-prone BB rats. The right panel gives the percentage (mean±1 SD) of positive cells in uninfected and LCMV-infected, diabetes-prone BB rats. P1, post infection.

reactive clones. In addition, our observations point to the ability of viruses, on the basis of their tropism for cells of the immune system, to regulate that system and its responses. Consistent with this view are our earlier studies that showed that persistent infection of New Zealand mice with LCMV or with a DNA virus, polyoma, markedly augments the occurrence and amount of antibodies to DNA and induces (in NZW mice) or aggravates (in NZB [NZB × W]F1) lupus-like disease (18). Alternatively, NZB and (NZB × W)F1 mice, which spontaneously develop this autoimmune disease, remain free from

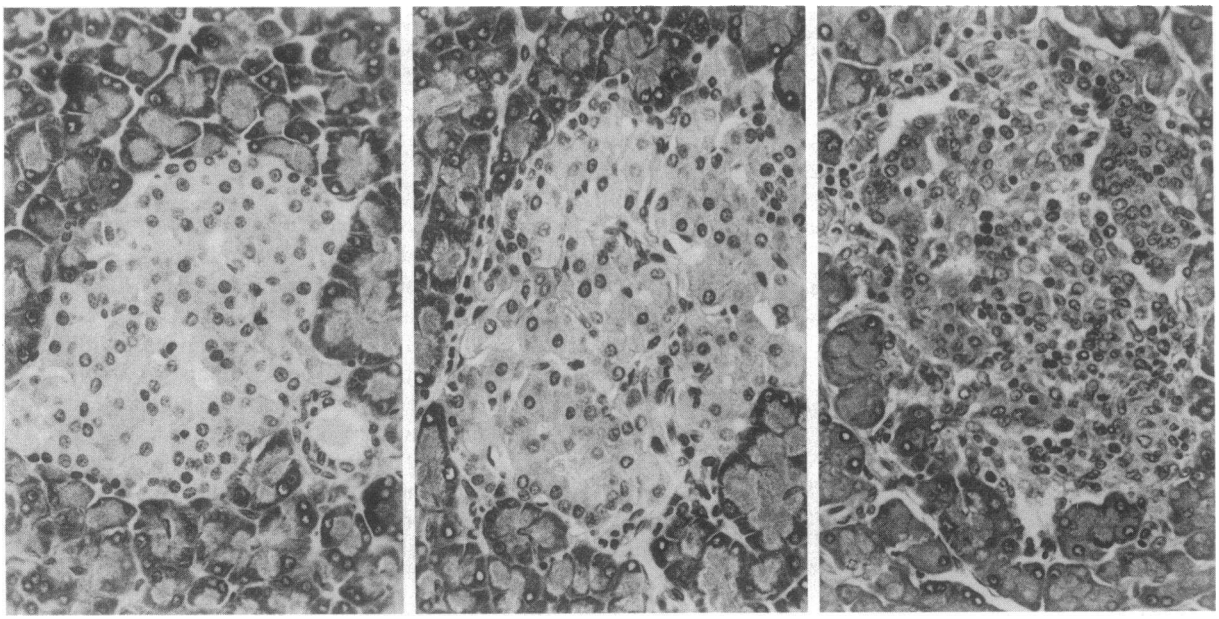

Figure 2. Pancreas morphology. Pancreata were fixed in Bouin's solution, embedded in paraffin, and sections were stained with hematoxylin and eosin. (*Left*) Normal islet from a diabetes-resistant BB rat. (*Middle*) Islet from an LCMV-infected, diabetes-prone BB rat, killed at 210 d of age. Minimal mononuclear cell infiltration is seen. (*Right*) Islet from a diabetic BB rat of recent onset, showing massive mononuclear cell infiltration and disruption of islet architecture.

lupus after infection with lactate dehydrogenase virus (LDV) (19). LDV preferentially infects macrophages and alters accompanying T lymphocyte functions (20–22). Recently, Inada and Mims have provided evidence that Ia molecules serve as receptors for LDV (21) and that infection with this virus aborts experimental allergic encephalitis (22), a disease caused by autoimmune responses against brain "self" proteins. Hence, understanding, in general, the mechanism by which viruses alter immune responses and specifically how LCMV decreases autoimmune responses of IDDM may offer a novel approach to specific immune therapy of IDDM and similar disorders of man.

Acknowledgments

Thomas Dyrberg was the recipient of a senior stipend from the University of Copenhagen, Denmark and Peter Schwimmbeck was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG).

This work was supported by National Institutes of Health grants AG-04342 and NS-12428 and by a NATO grant (T. Dyrberg) for international collaboration in research.

References

1. Marliss, E., A. Nakhooda, P. Poussier, and A. Sima. 1982. The diabetic syndrome of the "BB" Wistar rat: possible relevance to type I (insulin-dependent) diabetes in man. *Diabetologia.* 22:225–232.

2. Like, A. A., L. Butler, R. M. Williams, M. C. Appel, E. J. Weringer, and A. A. Rossini. 1982. Spontaneous autoimmune diabetes mellitus in the BB rat. *Diabetes.* 31:7–15.

3. Nakhooda, A., A. Like, C. Chappel, F. Murray, and E. Marliss. 1977. The spontaneously diabetic Wistar rat: metabolic and morphologic studies. *Diabetes.* 26:100–112.

4. Nakhooda, A., A. Like, C. Chappel, C.-N. Wei, and E. Marliss. 1978. The spontaneously diabetic Wistar rat (the "BB" rat): studies prior toetologia. 14:199–207.

5. Ahmed, R., A. Salmi, L. Butler, J. Chiller, and M. B. A. Oldstone. 1984. Selection of genetic variants of lymphocytic choriomeningitis virus in spleens of persistently infected mice. *J. Exp. Med.* 160:521–540.

6. McChesney, M., and M. B. A. Oldstone. 1987. Viruses perturb lymphocyte functions: selected principles characterizing virus-induced immunosuppression. *Annu. Rev. Immunol.* 5:279–304.

7. Butler, L., D. Guberski, and A. Like. 1983. Genetic analysis of the BB/W diabetic rat. *Can. J. Genet. Cytol.* 25:7–15.

8. Doyle, M. V., and M. B. A. Oldstone. 1978. Interactions between viruses and lymphocytes. I. *In vivo* replication of lymphocytic choriomeningitis virus in mononuclear cells during both chronic and acute viral infections. *J. Immunol.* 121:1262–1269.

9. Oldstone, M. B. A., R. S. Fujinami, A. Tishon, D. Finney, H. C. Powell, and P. W. Lampert. 1983. Mapping of the major histocompatibility complex and viral antigens on the plasma membrane of a measles virus infected cell. *Virology.* 127:426–437.

10. Klavinskis, L. S., and M. B. A. Oldstone. 1987. Lymphocytic choriomeningitis virus can persistently infect thyroid epithelial cells and perturb thyroid hormone function in mice. *J. Gen. Virol.* 68:1867–1873.

11. Southern, P. J., P. Blount, and M. B. A. Oldstone. 1984. Analysis of persistent virus infections by *in situ* hybridization to whole-mouse sections. *Nature (Lond.).* 312:555–558.

12. Valsamakis, A., Y. Riviere, and M. B. A. Oldstone. 1987. Perturbation of differentiated functions during viral infection *in vivo.* III. Lymphocytic choriomeningitis virus-induced growth hormone deficiency correlates with decreased growth hormone mRNA in persistently infected mice. *Virology.* 156:214–222.

13. Svenningsen, A., T. Dyrberg, H. Markholst, C. Binder, and A. Lernmark. 1986. Insulin release and pancreatic insulin are reduced in young prediabetic BB rats. *Acta Endocrinol.* 112:367–371.

14. Koevary, S., D. Williams, R. Williams, and W. J. Chick. 1985. Passive transfer of diabetes from BB/W to Wistar-Furth rats. *J. Clin. Invest.* 75:1904–1907.

15. Like, A., E. Kislauskis, R. Williams, and A. Rossini. 1982. Neonatal thymectomy prevents spontaneous diabetes mellitus in the BB/W rat. *Science (Wash. DC).* 216:644–646.

16. Like, A., A. Rossini, D. Guberski, M. Appel, and R. Williams. 1979. Spontaneous diabetes mellitus: reversal and prevention in the BB/W rat with antiserum to rat lymphocytes. *Science (Wash. DC).* 206:1421–1423.

17. Laupacis, A., C. Stiller, C. Gardell, P. Keown, J. Dupre, A. Wallace, and P. Thibert. 1983. Cyclosporin prevents diabetes in BB Wistar rats. *Lancet.* i:10–11.

18. Tonietti, G., M. B. A. Oldstone, and F. Dixon. 1970. The effect of induced chronic viral infections on the immunologic diseases of New Zealand mice. *J. Exp. Med.* 132:89–109.

19. Oldstone, M. B. A., and F. Dixon. 1972. Inhibition of antibodies to nuclear antigen and to DNA in New Zealand mice infected with lactate dehydrogenase virus. *Science (Wash. DC).* 175:784–786.

20. Oldstone, M. B. A., S. Yamazaki, A. Niwa, and A. Notkins. 1974. *In vitro* detection of cells infected with lactic dehydrogenase virus (LDV) by fluorescein-labeled antibody to LDV. *Intervirology.* 2:261–265.

21. Inada, T., and C. Mims. 1984. Mouse Ia antigens are receptor for lactate dehydrogenase virus. *Nature (Lond.).* 309:59–61.

22. Inada, T., and C. Mims. 1986. Infection of mice with lactic dehydrogenase virus prevents development of experimental allergic encephalomyelitis. *J. Neuroimmunol.* 11:53–58.