

New Approach to Immunotherapy Against Organ-specific Autoimmune Diseases with T Cell Receptor and Chemokine Receptor DNA Vaccines

Yoh Matsumoto*

Department of Molecular Neuropathology, Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan

Abstract: Organ-specific autoimmune diseases are characterized by the presence of relapse and remittance of the clinical signs, and last for a long period of time in most cases without an appropriate treatment. Immunopathologically, T cells that respond to organ-specific autoantigens play an important role in the development of inflammatory lesions in the target organ. These pathogenic T cells that had been activated by various stimuli including preceding infection infiltrate the target organ in an antigen-specific manner and break the homeostasis of the organ. Furthermore, they secrete a large number of pro-inflammatory cytokines and chemokines, which recruit by-stander inflammatory cells in the lesion. Although general immunosuppressive drugs such as corticosteroid and cyclosporine are effective in suppressing clinical signs and inflammation, immunospecific therapy is essential for the establishment of long-lasting remission or complete cure. In order to achieve effective immunospecific therapy, several groups have focused on two key molecules that are deeply involved in pathogenesis of autoimmune diseases. One is the T cell receptor (TCR) expressed on pathogenic T cells and the other is the cytokine and chemokine receptor expressed in the target organ. Another important aspect of this issue is the reagent that is used for the suppression of the function of the key molecules. So far, monoclonal antibodies, peptide vaccines and DNA vaccines are the major reagents used for immunosuppressive therapies.

In the present review, I introduce the results of immunotherapy obtained in my laboratory using TCR-based and chemokine receptor (chemoR)-based DNA in experimental autoimmune encephalomyelitis (EAE) and myocarditis (EAC) and discuss its effectiveness and pathomechanisms of immunosuppression. First, we administered DNA vaccines encoding pathogenic TCR V 8.2, 10 (to Lewis rats) and 15 (to DA rats) and observed that these vaccinations protected animals from the development of EAE [1]. Similar results were obtained in EAC [2]. Second, DNAs encoding several chemoRs were prepared and administered after the challenge to neutralize the function of chemokines that are highly upregulated in the lesions. It was demonstrated that these chemoR DNAs suppress the relapse of chronic relapsing EAE and block the progression of EAC to dilated cardiomyopathy (manuscripts submitted for publication). These findings clearly indicate that DNA vaccination can be a powerful tool for treatment of organ-specific autoimmune diseases.

INTRODUCTION

One of the characteristics of organ-specific autoimmune diseases is that the development of the diseases is closely associated with, or induced by, a particular type of T cells reactive to organ-specific antigens. For example, encephalitogenic T cell lines and clones generated from T cells of Lewis rats immunized with guinea pig myelin basic protein (MBP) are CD4-positive, belong to Th1 cells, and mainly use T cell receptor (TCR) V 8.2 [3-5]. These findings were obtained using the standard culture method [6] but non-V 8.2 encephalitogenic T cell lines could also be generated using different methods [7-9]. Therefore, the culture method has provided much information, but again there are limitations. To overcome these problems, we performed complementarity-determining region 3 (CDR3) spectratyping in which the TCR of oligoclonally expanded T cells are shown as dense bands compared with normal spectratypes. TCR showing oligoclonal expansion throughout the course of the disease is the most likely candidate for pathogenic TCR. Then, we determined

nucleotide and amino acid sequences of the CDR3 region of TCR clones derived from the spectratypes of interest to confirm whether there was a clonal expansion. Finally, we identified pathogenic TCR in the treatment experiments using mAbs or DNA vaccines. If TCRs screened by the series of analysis mentioned above were really pathogenic, then the treated animals would show no or mild clinical signs after being challenged with the disease-inducing antigen. The advantage of the new method is that it is applicable for the identification of the pathogenic TCR in a human autoimmune diseases and its TCR-based immunotherapy.

Another characteristic of organ-specific autoimmune diseases is recurrent formation of inflammatory lesions in the target organs. Although the pathogenesis of the lesion formation remains to be elucidated, it is assumed that target antigen-reactive T cells activated by several stimuli including preceding infection initially infiltrate the organ. Infiltrating T cells and resident cells activated by T cells secrete a variety of chemokines, which recruit by-stander T and other inflammatory cells that possess corresponding chemokine receptors on their surface. All these immunological events maintain inflammation in the organ for a certain period of time until these bioactive substances are subsided spontaneously or suppressed by immunotherapy. If chemokines responsible for the maintenance of inflammation

*Address correspondence to this author at the Department of Molecular Neuropathology, Tokyo Metropolitan Institute for Neuroscience, Musashidai 2-6, Fuchu, Tokyo 183-8526, Japan; Tel: +81-42-325-3881, ext. 4719; Fax:+81-42-321-8678; E-mail:matyoh@tmin.ac.jp

can be neutralized with therapeutic agents, then inflammation would be stopped during the active phase of autoimmune diseases.

In this review article, I introduce the findings obtained by TCR repertoire and chemokine analyses in experimental autoimmune encephalomyelitis (EAE) and carditis (EAC) and in multiple sclerosis (MS) patients and then show some results obtained by TCR- and chemokine-targeted DNA therapy in animal models.

CHARACTERIZATION OF PATHOGENIC T CELL RECEPTOR (TCR) IN HUMAN AUTOIMMUNE DISEASES AND THEIR ANIMAL MODELS

EAE

EAE is one of the most characterized animal models for human autoimmune diseases. It is well known that EAE-inducing T cells bear CD4 molecules and use a limited number of α and β chains of the T cell receptor (TCR) [10]. Furthermore, the CDR3 region of TCR of cultured encephalitogenic T cell clones, is rather short and some amino acid residues are conservatively preserved [4, 5]. In Lewis rats, encephalitogenic T cell clones established from guinea pig MBP-immunized animals mainly use V 8.2 [3]. Moreover, others [12] and we [11] have demonstrated by immunohistochemical and flow cytometric studies that V 8.2-positive T cells infiltrate the CNS at the early stage of EAE and become a predominant population throughout the course of EAE. However, V 8.2 is not the sole TCR used by encephalitogenic T cells. Non-V 8.2 EAE-associated T cells could be generated and MBP-reactive T cell lines bearing V 3.3 [13], V 6 [14, 15], V 10 [9] and V 18 [7] were shown to be encephalitogenic.

It is, however, difficult to judge which V -positive (V 3.3, V 6, V 8.2, V 10 or V 18 in the above cases) encephalitogenic T cells play the central role in the development of EAE using the conventional method. To address this issue, we performed CDR3 spectratyping analysis using PBL and spinal cord T cells and demonstrated marked V 8.2 spectratype expansion throughout the course of acute EAE [16]. Then, PCR products were extracted from V 8.2 spectratypes showing expansion at early, peak and recovery stages of acute EAE and their CDR3 sequence was determined after cloning. In this case, the DSSYEQYFGPG sequence was most predominant at all the stages examined and the ASQNTLFFGAG sequence was frequently found, indicating that the TCR repertoire of expanded V 8.2 spectratype remained unchanged throughout the course of acute EAE [16, 17].

DA rats are another autoimmune disease-susceptible strain. After immunization with MBP, rats develop acute EAE [18]. However, encephalitogenic epitopes in the MBP molecules for the DA and Lewis strains are different from each other. While MBP63-81 causes severe EAE in DA rats but not in Lewis rats, MBP87-99 which is encephalitogenic for Lewis is inactive in DA rats [18]. There are also differences between these two strains. DA rats develop clinical EAE after immunization with encephalitogenic antigens, not only with complete Freund adjuvant (CFA), but

also with incomplete Freund adjuvant; while Lewis rats develop EAE only after immunization with antigen/CFA [19]. To identify clonally or oligoclonally expanded T cells in DA rats, we screened T cells that infiltrated the spinal cord by CDR3 spectratyping. In sharp contrast to Lewis rats, a variety of T cell receptors showed expansion in MBP-immunized DA rats. However, when an encephalitogenic peptide, the 62-75 residue of the MBP molecule, was immunized, spinal cord T cells showed spectratype expansion of V s including V 10 and V 15 [1]. These findings indicate that TCRs showing oligoclonal expansion vary, depending on the strain of rats even immunized with the same antigen.

Other Animal Models

Experimental autoimmune myocarditis (EAC) is inducible in Lewis rats by immunization with cardiac myosin. Recently, we observed using CDR3 spectratyping that oligoclonal expansion of V 8.2 and V 10 persists throughout the course of the disease [20]. These V s are proved to be pathogenic TCR from the evidence that depletion or suppression of V 8.2- and V 10-positive T cells resulted in significant protection from the disease development (see below).

Multiple Sclerosis and other Human Diseases

It is believed that multiple sclerosis (MS) is an autoimmune disease mediated by neuroantigen-reactive T cells [21, 22]. However, there is no consensus with regard to the TCR usage by T cells associated with the development of MS. Initially, Wucherpfennig *et al.* examined MBP-specific T cell lines and found that V 17 and, to a lesser extent, V 12 were frequently used [23], while Kotzin *et al.* found the preferential usage of V 5.2 and V 6.1 by MBP-specific T cell clones [24]. Later trials failed to confirm the preferential usage of V 5.2 by MBP-specific T cell lines [25, 26]. Other groups insisted that although a particular type of TCR was predominantly used by MBP-specific T cell clones from a single MS patient, it varies from patient to patient, suggesting individual-specific TCR restriction [27, 28]. The reasons for the diverse findings may be partly attributable to the difference in the method used to establish MBP-specific T cell lines and clones.

To avoid bias produced during culture, we applied CDR3 spectratyping analysis to identify pathogenic TCR using PBL and cerebrospinal fluid (CSF) cells taken from MS patients at various stages and from healthy subjects using CDR3 spectratyping. We have carried out an overall TCR chain repertoire analysis for the first time and demonstrated that V 5.2 and, to a lesser extent, V 24 spectratypes are expanded more frequently than other V s in MS patients (Fig. 1) [29]. Sequence analysis of the CDR3 region of spectratype-derived TCR clones revealed that the predominant TCR clone was different from patient to patient but that similar results were obtained in a patient examined at different time points. More importantly, examination of CSF T cells and longitudinal studies of PBL from selected patients revealed that V 5.2 expansion was detectable in approximately 70% of patients examined. These findings

suggest that V 5.2 spectratype expansion is associated with the development of MS and that TCR-based immunotherapy can be applicable to MS patients if the TCR activation pattern of each patient is determined at different stages of the disease.

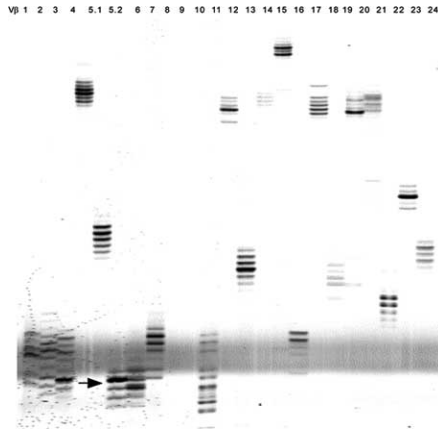


Fig. (1). CDR3 spectratyping analysis of PBL from an MS patient. V 5.2 spectratype expansion (arrow) with additional spectratype expansion such as V 23 is noted.

We also examined PBL from patients with Guillain-Barre syndrome (GBS) and Fisher syndrome (FS) to identify the pathogenic TCR. In this case, we did not observe any preferential V spectratype expansion among the patients. Subgrouping the patients by *Campylobacter jejuni* serology and anti-ganglioside IgG antibodies also failed to detect particular spectratype gene usage. These findings suggest that although T cells are involved in the pathogenesis of GBS and FS, TCRs used by the T cells vary from patient to patient [30].

TCR-BASED IMMUNOTHERAPY WITH SYNTHETIC PEPTIDE AND DNA VACCINES

As organ-specific, autoimmune diseases are mediated by T cells reactive to organ-specific antigens, so therapy targeting such T cells has long been considered as a primary immunotherapy. Based on the findings obtained using attenuated T cells as a vaccine [31], the TCR peptide vaccination therapy was developed. Administration of synthetic peptides corresponding to the CDR2 or CDR3 region of encephalitogenic TCR, but not of irrelevant TCR peptides, was reported to protect animals from EAE upon subsequent challenge with encephalitogenic antigen [32, 33]. However, further studies [34-36] failed to confirm the clear suppressive effects of TCR peptide vaccination. One reason for this discrepancy is that injected peptides would be degraded so rapidly that sufficient amounts of anti-TCR antibodies and cytotoxic T cells are not induced in some cases.

TCR-based DNA vaccine therapy for EAE was first performed by Steinman's group [37]. They showed that vaccination with DNA encoding V 8.2 protected mice from EAE and observed upregulation of Th2 cytokines in vaccinated animals. Based on these findings, they concluded that this Th2 cytokine deviation is the major protective effect of DNA vaccines. We have extended Steinman's work and

tried to prevent another organ-specific autoimmune disease, EAC, whose pathomechanisms are poorly understood. CDR3 spectratyping analysis revealed that oligoclonal expansion of V 8.2 and V 10 persists throughout the course of the disease. However, it is impossible at present to establish myosin-reactive carditogenic T cell lines and to examine their TCR usage because cardiac myosin is self-aggregatable and thus is not suitable for *in vitro* stimulation of T cells [20]. Alternatively, we tried to identify pathogenic TCR by treatment experiments. If candidate TCRs screened by CDR3 spectratyping are really pathogenic TCRs, then immunotherapy targeted candidate TCR would protect animals from the development of EAC. Combination therapy targeting V 8.2 and V 10, but not targeting either alone, significantly suppressed autoimmune inflammation in the heart. These findings demonstrate that the determination of candidate TCR genes by CDR3 spectratyping and subsequent immunotherapy serves not only to elucidate the pathomechanism, but also to provide a systematic therapeutic strategy for T cell-mediated autoimmune diseases.

Table 1. Immunotherapy of EAC with DNA Vaccines. Lewis rats were Pretreated by Injection of 0.75% Bupivacaine (1 μ l/g Body Weight) in the Tibialis Anterior Muscle and 100 μ g in 50 μ l DNA was Injected Twice at 2-week Intervals and Challenged for EAC. On Day 14 Post-immunization, Hearts were Removed and Processed for Hematoxylin-eosin Staining.

DNA	Incidence	Histological grade
V 8.2	4/4	3.0 \pm 0.7
V 10	3/4	2.3 \pm 1.3
V 8.2 + V 10	2/4	0.9 \pm 0.9
empty vector	4/4	3.0 \pm 1.1

In MBP peptide-induced EAE in DA rats, there was a frequent spectratype expansion of V 10 and V 15 as described above. Injection of V 10 and V 15 DNA vaccines completely suppressed the development of EAE in 30% of immunized rats and significantly reduced the severities of clinical EAE in the rest [1]. Thus, TCR-based DNA vaccine therapy could be effective for the treatment of autoimmune diseases whose pathogenic T cells are well characterized in terms of TCR usage. This therapy is applicable for not only animal models but also human diseases.

CYTOKINE AND CHEMOKINE PROFILES IN AUTOIMMUNE DISEASES

In the target organ, inflammatory foci mainly consist of infiltrating T cells and macrophages. Pathogenic T cells infiltrate the organ by recognizing autoantigens in the organ. These T cells and resident cells stimulated by the T cells secrete variety of cytokines and chemokines and recruit bystander T cells and macrophages [38]. Therefore, it is very important to determine cytokines and chemokines that are closely associated with immunological events that take place

during the course of autoimmune diseases. Here, I introduce two samples of cytokine and chemokine analysis, which we have done using the EAE and EAC models.

One of characteristics of autoimmune diseases is the presence of clinical and histopathological relapse of the disease. Using the EAE model, many groups investigated the mechanism of the relapse and reported factors that are related to this phenomenon. Lehmann *et al.* demonstrated that immunization of mice with MBP initially induced the proliferative responses only to the immunodominant epitope of MBP but that the responses to several cryptic epitopes were found in mice with chronic EAE [39]. These findings suggest that diversification of the autoreactive T cell repertoire due to epitope spreading is closely associated with the relapse of the disease. In addition to these investigations, special efforts were made to identify bioactive substances involved in the process of the disease relapse. Consequently, it was demonstrated that IL-12 [40], monocyte chemoattractant protein-1 (MCP-1) [41] and perforin [42] play a pivotal role in this process. We previously investigated the factors involved in the relapse of EAE and found that upregulation of TNF- α , IFN- γ and MCP-1, alone or in combination, in the CNS is closely associated in the development of the disease relapse by comparing the difference between acute and chronic relapsing (CR) EAE induced in DA and Lewis rats [43, 44]. However, in both cases, encephalitogenic antigens and protocols used for induction of the two types of EAE were not exactly the same so that we could not exclude the possibility that difference in the levels of the above cytokines and chemokines is attributable to the difference in the induction protocols and not to the difference in the presence or absence of the relapse. Recently, we were able to induce acute and CR EAE in DA rats using the same antigen and immunization protocol. Only one difference seen was in the dose of antigen administered. While rats immunized with a relatively low dose of encephalitogenic antigen developed acute monophasic EAE, a large proportion of rats immunized with a high dose exhibited CR EAE. By comparing these two types of EAE induced in the same manner except the dose of immunizing antigen, we could identify factors that play a pivotal role in the development of EAE relapse. Consequently, it was found that gamma interferon (IFN- γ) and interferon-inducible protein-10 (IP-10) in the spinal cord were significantly elevated during the first and second attacks, respectively, of CR EAE than at the peak of acute EAE (our unpublished observation).

Another sample is EAC and dilated cardiomyopathy (DCM). Severe EAC and subsequent DCM were successfully produced in Lewis rats by immunization with cardiac C-protein. After immunization, a considerable number of immunized animals died of acute cardiac failure due to severe inflammation and all the survived rats showed typical DCM characterized by the presence of ventricular dilatation and extensive fibrosis. Immunopathological and chemokine analysis during the acute phase revealed that there were marked macrophage infiltration with myocyte necrosis and upregulation of MCP-1 and IP-10. We are able to determine the critical role of these chemokines by treatment experiments (manuscript submitted for publication).

IMMUNOTHERAPIES TARGETING CHEMOKINES AND THEIR RECEPTORS WITH FUSION PROTEIN AND DNA

Immunotherapy, targeting cytokines and chemokines, is well established in rheumatoid arthritis. A recombinant tumor necrosis factor:Fc fusion protein, etanercept, has shown to be safe and well-tolerated, and provides significantly greater improvement in the inflammatory symptoms of rheumatoid arthritis [45, 46].

As shown in the previous chapter, chemokines and chemokine receptor (chemoR) play a critical role in the maintenance of autoimmune inflammation in the target organ other than rheumatoid arthritis. This also suggests that immunotherapies, targeting these molecules, can be developed to stop the progression of the diseases. In EAC, immunopathological examination and chemokine analysis of the cardiac lesion strongly suggest that MCP-1 and IP-10, chemoattractants for T cells and macrophages, play an essential role in the formation and maintenance of EAC lesions. To confirm their role and establish immunotherapy targeting chemokines to control autoimmune processes, we prepared DNAs encoding the binding sites of CC chemokine receptor 2 (CCR2) and CXC chemokine receptor 3 (CXCR3), which are receptors for MCP-1 and IP-10, respectively. Rats were immunized with cardiac C-protein; and chemoR DNAs, alone or in combination were injected from day 1 to day 27 post-immunization and were sacrificed for histological evaluation at 6 weeks. Administration of DNAs encoding CCR2 and CXCR3, which inhibit cell migration induced by the above chemokines *in vitro*, to immunized rats, prevented the disease progression and rescued animals from death. These findings demonstrate that immunotherapy targeting chemokines is a powerful tool for the control of EAC and DCM (manuscript submitted for publication).

Rats with CR EAE were treated in the same strategy. Unlike EAC, chemoR DNA therapy did not suppress the first attack. However, relapse of the disease was prevented in some animals with this treatment. Although precise reasons for this remain unknown, it was suggested that cytokines and chemokines other than MCP-1 and IP-10 play an important role in the first attack of chronic relapsing EAE (unpublished observation).

CONCLUDING REMARKS

In this review, I summarized recent results regarding the pathogenesis of human autoimmune diseases and their animal models and TCR and chemokine-based gene therapy using non-viral vectors. The major problem of the use of non-viral vector for gene delivery is low efficiency in transfer of the gene into cells. However, recent progress in technology in this field is marked. Several improvements in making liposomes and polymers have greatly increased the efficiency in transfer of genes into cells *in vitro* and *in vivo*. There are also new technologies in preventing the transferred gene from degradation in endosomes and in potentiating the gene transfer into the nucleus. All these technologies will greatly increase the efficacy of gene therapy against autoimmune diseases

ABBREVIATIONS

CDR3	=	Complementarity determining region 3
CFA	=	Complete Freund adjuvant
CNS	=	Central nervous system
CSF	=	Cerebrospinal fluid
DCM	=	Dilated cardiomyopathy
EAE	=	Experimental autoimmune encephalomyelitis
EAC	=	Experimental autoimmune myocarditis
MBP	=	Myelin basic protein
MOG	=	Myelin oligodendrocyte glycoprotein
MS	=	Multiple sclerosis
PBL	=	Peripheral blood lymphocytes
TCR	=	T cell receptor

REFERENCES

- Miyakoshi, A.; Yoon, W. K.; Jee, Y. and Matsumoto, Y. (2003) *J. Immunol.*, **170**, 6371-6378.
- Matsumoto, Y. (2000) *J. Neuroimmunol.*, **110**, 1-12.
- Burns, F. R.; Li, X.; Shen, N.; Offner, H.; Chou, Y. K.; Vandenbark, A. A. and Heber-Katz, E. (1989) *J. Exp. Med.*, **169**, 27-39.
- Gold, D. P.; Offner, H.; Sun, D.; Wiley, S.; Vandenbark, A. A. and Wilson, D. B. (1991) *J. Exp. Med.*, **174**, 1467-1476.
- Zhang, X. and Heber-Katz, E. (1992) *J. Immunol.*, **148**, 746-752.
- Sedgwick, J. D.; MacPhee, I. A. M. and Puklavec, M. (1989) *J. Immunol. Methods*, **121**, 185-196.
- Sun, D.; Hu, X. and Coleclough, C. (1995) *Eur. J. Immunol.*, **25**, 69-74.
- Urban, J. L.; Kumar, V.; Kono, D. H.; Gomez, C.; Horvath, S. J.; Clayton, J.; Ando, D. G.; Sercarz, E. E. and Hood, L. (1988) *Cell*, **54**, 577-592.
- Gold, R.; Giegerich, G.; Hartung, H. and Toyka, K. V. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 5850-5854.
- Burns, J.; Rosenzweig, A.; Zweiman, B.; Moskovitz, A. and Lisak, R. (1984) *J. Immunol.*, **132**, 2690-2692.
- Tsuchida, M.; Matsumoto, Y.; Hirahara, H.; Hanawa, H.; Tomiyama, K. and Abo, T. (1993) *Eur. J. Immunol.*, **23**, 2399-2406.
- Lannes-Vieira, J.; Gehrman, J.; Kreutzberg, G. W. and Wekerle, H. (1994) *Acta Neuropathol.*, **87**, 435-442.
- Chan, A.; Gold, R.; Giegerich, G.; Herrmann, T.; Jung, S.; Toyka, K. V. and Hartung, H. (1999) *J. Neurosci. Res.*, **58**, 214-225.
- Offner, H.; Vainiene, M.; Gold, D. P.; Celnik, B.; Wang, R.; Hashim, G. A. and Vandenbark, A. A. (1992) *J. Immunol.*, **148**, 1706-1711.
- Sun, D.; Gold, D. P.; Smith, L.; Brostoff, S. and Coleclough, C. (1992) *Eur. J. Immunol.*, **22**, 591-594.
- Kim, G.; Tanuma, N.; Kojima, T.; Kohyama, K.; Suzuki, Y.; Kawazoe, Y. and Matsumoto, Y. (1998) *J. Immunol.*, **160**, 509-513.
- Kim, G.; Kohyama, K.; Tanuma, N.; Arimoto, H. and Matsumoto, Y. (1998) *J. Immunol.*, **161**, 6993-6998.
- Stepaniak, J. A.; Wolf, N. A.; Sun, D. and Swanborg, R. H. (1997) *J. Neuroimmunol.*, **78**, 79-85.
- Lenz, D. C.; Wolf, N. A. and Swanborg, R. H. (1999) *J. Immunol.*, **163**, 1763-1768.
- Matsumoto, Y.; Jee, Y. and Sugisaki, M. (2000) *J. Immunol.*, **164**, 2248-2254.
- Ota, K.; Matsui, M.; Milford, E. L.; Mackin, G. A.; Weiner, H. L. and Hafler, D. A. (1990) *Nature*, **346**, 183-187.
- Pette, M.; Fujita, K.; Wilkinson, D.; Altmann, D. M.; Trowsdale, J.; Giegerich, G.; Hinkkanen, A.; Epplen, J. T.; Kappos, L. and Wekerle, H. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 7968-7972.
- Wucherpfennig, K. W.; Ota, K.; Endo, N.; Seidman, J. G.; Posenszweig, A.; Weiner, H. L. and Hafler, D. A. (1990) *Science*, **248**, 1016-1019.
- Kotzin, B. L.; Karuturi, S.; Chou, Y. K.; Lafferty, J.; Forrester, J. M.; Better, M.; Nedwin, G. E.; Offner, H. and Vandenbark, A. A. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 9161-9165.
- Meini, E.; Weber, F.; Drexler, K.; Morelle, C.; Ott, M.; Saruhan-Direskeneli, G.; Goebels, N.; Ertl, B.; Jechart, G.; Giegerich, G.; Schoenbeck, S.; Bannwarth, W.; Wekerle, H. and Hohlfeld, R. (1993) *J. Clin. Invest.*, **92**, 2633-2643.
- Afshar, G.; Muraro, P. A.; McFarland, H. F. and Martin, R. (1998) *J. Neuroimmunol.*, **84**, 7-13.
- Ben-Nun, A.; Liblau, R. S.; Lehmann, D.; Tournier-Lasserre, E.; Rosenzweig, A.; Jingwu, Z.; Raus, J. C. M. and Bach, M.-A. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 2466-2470.
- Vandevyver, C.; Mertens, N.; van den Elsen, P.; Medaer, R.; Raus, J. and Zhang, J. (1995) *Eur. J. Immunol.*, **25**, 958-968.
- Matsumoto, Y.; Yoon, W. K.; Jee, Y.; Fujihara, K.; Mitsu, T.; Sato, S.; Nakashima, I. and Itoyama, Y. (2003) *J. Immunol.*, **170**, 4846-4853.
- Koga, M.; Yuki, N.; Tsukada, Y.; Hirata, K. and Matsumoto, Y. (2003) *J. Neuroimmunol.*, in press.
- Ben-Nun, A.; Wekerle, H. and Cohen, I. R. (1981) *Nature*, **292**, 60-61.
- Vandenbark, A. A.; Hashim, G. and Offner, H. (1989) *Nature*, **341**, 541-544.
- Howell, M. D.; Winters, S. T.; Olee, T.; Powell, H. C.; Carlo, D. J. and Brostoff, S. V. (1989) *Science*, **246**, 668-670.
- Desquenue-Clark, L.; Esch, T. R.; Otvos, L. Jr. and Heber-Katz, E. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 7219-7223.
- Kawano, Y.; Sasamoto, Y.; Kotake, S.; Thurau, S. R.; B. Wiggert and Gery, I. (1991) *Cur. Eye Res.*, **10**, 789-795.
- Tanuma, N.; Abe, S.; Shin, T.; Kojima, T.; Ishihara, Y. and Matsumoto, Y. (1996) *Cell. Immunol.*, **168**, 85-90.
- Waisman, A.; Ruiz, P.; Hirschberg, D. L.; Gelman, A.; Oksenberg, J. R.; Brocke, S.; Mor, F.; Cohen, I. R. and Steinman, L. (1996) *Nature Med.*, **2**, 899-905.
- Sallusto, F.; Mackay, C. R. and Lanzavecchia, A. (2000) *Ann. Rev. Immunol.*, **18**, 593-620.
- Lehmann, P. V.; Forsthuber, T.; Miller, A. and Sercarz, E. E. (1992) *Nature*, **358**, 155-157.
- Smith, T.; Hewson, A. K.; Kingsley, C. I.; Leonard, J. P. and Cuzner, M. L. (1997) *Am. J. Pathol.*, **150**, 1909-1917.
- Karpus, W. J.; Lukacs, N. W.; McRae, B. L.; Strieter, R. M.; Kunkel, S. L. and Miller, S. D. (1995) *J. Immunol.*, **155**, 5003-5010.
- Malipeiro, U. V.; Frei, K.; Spanaus, K.; Agresti, C.; Lassmann, H.; Hahne, M.; Tschopp, J.; Eugster, H. and Fontana, A. (1997) *Eur. J. Immunol.*, **27**, 3151-3160.
- Tanuma, N.; Shin, T.; Kogure, K. and Matsumoto, Y. (1999) *J. Neuroimmunol.*, **96**, 73-79.
- Jee, Y.; Yoon, W. K.; Okura, Y.; Tanuma, N. and Matsumoto, Y. (2002) *J. Neuroimmunol.*, **128**, 49-57.
- Moreland, L. W.; Baumgartner, S. W.; Schiff, M. H.; Tindall, E. A.; Fleischmann, R. M.; Weaver, A. L.; Ettliger, R. E.; Cohen, S.; Koopman, W. J.; Mohler, K.; Widmer, M. B. and Blosch, C. M. (1997) *N. Engl. J. Med.*, **337**, 141-7.
- Weinblatt, M. E.; Kremer, J. M.; Bankhurst, A. D.; Bulpitt, K. J.; Fleischmann, R. M.; Fox, R. I.; Jackson, C. G.; Lange, M. and Burge, D. J. (1999) *N. Engl. J. Med.*, **340**, 253-9.