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Virus-specific and autoreactive T cell lines isolated from cerebrospinal fluid of a patient with chronic rubella panencephalitis

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Summary

Using a recently described technique for expanding of human T lymphocyte populations from cerebrospinal fluid (CSF), we investigated the local cellular immune response in a patient with chronic rubella panencephalitis. A total of 328 T cell lines (TCLs) was established by seeding CSF cells at limiting dilution into histoplates in the presence of irradiated feeder cells and phytohemagglutinin (PHA)-containing conditioned medium. 80% of TCLs expressed the CD4+CD8- phenotype, 5% the CD4-CD8+ phenotype and 15% of TCLs contained different proportions of CD4+ and CD8+ cells. Of 191 TCLs analyzed, 85 were cytotoxic, as shown by their lectin-dependent cytotoxicity against allogeneic uninfected target cells. Eight of them demonstrated specificity for the autologous, rubella virus-infected target cells. When tested for antigen-specific proliferative activity, 26 TCLs responded to rubella antigen, 16 TCLs reacted to myelin basic protein (MBP), four TCLs to proteolipid protein (PLP), four to galactocerebrosides and two to actin. Fourteen out of 16 MBP-specific TCLs also responded, to a minor degree, to rubella antigen and/or actin. The results showed that the persisting rubella infection had given rise to autoreactive T cells. Virus-induced autoreactivity to brain antigens may be an important pathogenetic mechanism in other chronic inflammatory disorders of the CNS.

Introduction

Until recently, studies on cell-mediated immunity in inflammatory brain disorders were severely hampered by the small number of cells available in the cerebrospinal fluid (CSF). This problem has
been overcome by progress in modern tissue culture techniques and the use of interleukin-2 (IL-2) as a T cell growth factor.

By using IL-2 it is now possible to expand and analyze functional T cell populations in the CSF. We have used this technique successfully in acute virus infections of the CNS such as mumps meningitis and measles encephalitis (Fleischer and Kreth, 1983a, b). The results showed that a high proportion of CSF T cells were specific for the infecting virus. Virtually all cytotoxic T cells were found to be virus-specific and restricted by class I human leukocyte antigens (HLA).

Recently, we had the opportunity to study a patient with progressive rubella panencephalitis (PRP). This rare disease has been described as a late sequel of either congenital or postnatal rubella infection (Lebon and Lyon, 1974; Townsend et al., 1975; Weil et al., 1975; Wolinsky et al., 1976). After a latent period of many years, patients present with a gradually deteriorating syndrome characterized by intellectual impairment, seizures, myoclonus, dementia, choreiform movements and cerebellar ataxia. Analysis of the CSF shows high levels of IgG with oligoclonal bands specific for rubella virus. The disease resembles subacute sclerosing panencephalitis (SSPE) to some extent; there are, however, some distinct differences. For instance, a moderate lymphoid pleocytosis is frequently observed in patients with PRP. Rubella virus has been recovered from brain tissues, with and without co-cultivation techniques, and also from infected lymphocytes (Cremer et al., 1975; Wolinsky et al., 1979). Viral antigens have, however, not been detected by immunofluorescence in brain sections using immunofluorescence, and the exact site and molecular biology of the persistent rubella virus is unknown.

The fact that rubella virus is a non-cytopathogenic agent suggests that PRP is essentially an immunologically mediated disorder, perhaps an immune complex-mediated disease (Waxham et al., 1984).

The aim of the present study was to analyze the specificity of the T cells present in the CSF during this chronic virus infection.

Patient and methods

Case report

The patient, a girl, was born in 1970. There were no stigmata of congenital rubella syndrome. She had measles at 2 years and rubella at 8 years of age. She developed normally until 11 years of age when she attracted attention because of deficits in school performance. Two months later, when admitted to the University Children's Hospital in Graz, Austria, she presented with disorientation, adynamia, aggressive behaviour and choreiform movements. During the following weeks her neurological findings worsened. Two grand mal seizures occurred. The electroencephalogram (EEG) was severely altered with diffuse dysrhythmia, slow-wave activity with bursts of theta and delta waves and some interspersed sharp-wave complexes.

CSF analysis showed a pleocytosis of 120 lymphoid cells/μl, a total protein concentration of 33 mg/dl and a markedly elevated gammaglobulin fraction. CSF antibody titres for measles, herpes simplex type 1, varizella zoster, mumps, RS, cytomegal- and adenoviruses were negative, but high levels of rubella antibodies were found. Rubella-specific hemagglutination inhibiting (HI) antibody titres were 1:512 in CSF and >1:2048 in serum. Moreover, oligoclonal bands specific for rubella virus mainly of kappa light chain type were demonstrated by imprint immunofixation and immunoblotting of electrophoresed CSF (kindly determined by Dr. B. Vandvik, Department of Neurology, Oslo, Norway, and by Dr. R. Dörries, Institute of Virology, University of Würzburg, F.R.G.). Rubella-specific IgM was not detected. Attempts to isolate infectious virus from CSF and peripheral blood mononuclear cells were unsuccessful. Based on these findings, a diagnosis of postnatally acquired rubella panencephalitis was made.

This disease is usually a progressive and fatal inflammatory disorder of the CNS. Surprisingly, in this patient, almost all neurological deficits gradually disappeared during the following months.

Now, 6 years later, she shows almost normal school performance with only slight intellectual deficits. However, CSF pleocytosis (ranging from 30 to 50 lymphoid cells/μl) and raised CSF gamma globulin levels (10 to 12 mg/dl IgG) with pronounced rubella-specific oligoclonal bands are still present.

Cells

CSF and venous blood were obtained from the patient during a short visit to the Children's Hospital of Würzburg University. At that time the girl had been in clinical remission for about 2 years. CSF lymphocytes were isolated from 12 ml of freshly drawn CSF containing 33 lymphoid cells/μl as described (Kreth et al., 1982). Peripheral blood mononuclear leukocytes (PBL) were
prepared by sedimentation on Ficoll-Isopaque. CSF lymphocytes and PBL were cryo-preserved at $1 \times 10^5$ and $5 \times 10^6$ cells/ml, respectively, and stored in liquid nitrogen. All blood and CSF samples were obtained with the parents' informed consent.

HLA-A, -B, -C and -DR typing was kindly performed by Dr. S.F. Goldmann, Department of Transplantation Immunology, Red Cross Blood Service, Ulm, F.R.G. The HLA type of the patient was: Aw23, A26, Bw49, Bw61, Cw2, DR1, DR2.

**Generation of T cell colonies from CSF**

Cloning of T lymphocytes was performed by limiting dilution in histoplates as described recently (Fleischer and Kreth, 1983a, b). Briefly, CSF cells were thawed and preincubated for 2 h at 37°C in Hepes-buffered RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine and 50 μg/ml gentamicin (Flow Laboratories, Meckenheim, F.R.G.). This medium will be referred to hereafter as complete medium. The viability of the cells as determined by trypan blue exclusion was usually greater than 90%, and the recovery was about 50–70% of the cells frozen.

Cells were seeded at 1, 3, 10 and 30 cells/well in 50% crude mitogen-containing conditioned medium in the presence of irradiated (6000 R) allogeneic PBL (1 x 10^6/ml) in histoplates (Greiner, Nürtingen, F.R.G.). At least 300 wells were seeded at each cell concentration. T cell growth factor (IL-2)-conditioned medium was obtained from 24 h phytohemagglutinin (PHA)-stimulated PBL from young male donors as described (Lotze and Rosenberg, 1981). Growth was determined by microscopy on days 8, 10, 12, 14 and 18 after seeding. Growing colonies were transferred to larger wells and expanded in medium containing 20% lectin-free IL-2 (Lymphocult T-LF; Biotest, Dreieichenhain, F.R.G.) until functional tests became possible.

**Immunofluorescent staining of surface antigens**

OKT3, OKT4, OKT8 and OKIa monoclonal antibodies were purchased from Ortho Pharmaceutical Co. (Heidelberg, F.R.G.). Rubella-specific monoclonal antibodies were kindly donated by Dr. R. Tedder (Department of Virology, Middlesex Hospital, London, U.K.). Fluorescein-conjugated goat anti-mouse antibody (Tago, Burlingame, CA, U.S.A.) was used as a second antibody for indirect immunofluorescent staining.

Rubella viral antigens were also detected using a specific polyclonal sheep antiserum and fluorescein-labeled rabbit IgG to sheep immunoglobulins (Dakopatts, Denmark). Prior to use, the sheep antiserum to rubella virus was extensively absorbed with uninfected rabbit kidney (RK-13) cells.

**Antigens**

The Judith strain of rubella virus was grown in continuous baby hamster kidney cells (BHK-21 cells). Supernatant fluids were harvested daily and replaced with fresh maintenance medium. The infectivity of each harvest was determined by titration in RK-13 cells. The maximum titer of virus ($10^7$ TCID₅₀/0.1 ml) was obtained 3 days after infection. The same stock of virus was used in all cytotoxicity assays.

Rubella virus used in lymphoproliferative studies was prepared as described (Al-Nakib et al., 1975). The antigen preparations had hemagglutinating titres between 256 and 1024. Control antigen was prepared by the same method using uninfected BHK-21 cells.

The Enders strain of mumps virus was grown on allantoic membranes of 8-day-old embryonated eggs and concentrated by ultracentrifugation (Kreth et al., 1982). Concentrated mumps virus stocks had hemagglutinating titres between 512 and 2048.

Human myelin basic protein (MBP) was prepared according to Banik and Davison (1973) and kindly provided by Dr. A. Kohlschütter, University Children’s Hospital, Hamburg, F.R.G.

Galactocerebrosides (type II) and actin were purchased from Sigma (Taufkirchen, F.R.G.). Galactocerebrosides were dissolved in 50% ethanol and diluted in phosphate-buffered saline to a final concentration of 5%. The suspension was always sonicated prior to use.

Proteolipid protein (PLP) prepared according to Stoffel et al. (1983) was a generous gift from Dr. C. Linington, Max-Planck-Gesellschaft, Klinische Forschungsgruppe für Multiple Sklerose, Würzburg, F.R.G.
Preparation of target cells

Target cells were prepared from autologous and allogeneic PBL of known HLA type. 5–10 × 10^6 freshly isolated or cryopreserved and thawed PBL were infected with the Judith strain of rubella virus at a multiplicity of infection (MOI) of 1 in 1.0 ml serum-free RPMI medium. After 90 min incubation at room temperature, cells were resuspended in 10 ml complete medium and incubated in 50 ml upright tissue culture flasks (Greiner, Nürtingen, F.R.G.) at 37°C in humidified 5% CO\textsubscript{2}/air. 10 μl of undiluted PHA-P (Difco Laboratories, Detroit, MI, U.S.A.) was added after 24 h, and the cells allowed to incubate for a further 72 h.

On the day of testing, 60–80% of virus-infected PHA blasts expressed rubella antigens on their surface as demonstrated by immunofluorescent staining with either specific monoclonal or polyclonal antibodies. Moreover, infectious virus could be rescued from supernatant fluids using continuous green African monkey kidney (Vero) and RK-13 cell cultures.

Cytotoxicity assay

Virus-infected and uninfected target cells were labeled with 100–200 μCi Na\textsuperscript{51}CrO\textsubscript{4} (New England Nuclear, Dreieich, F.R.G.). A 5 h \textsuperscript{51}Cr-release assay was performed in V-shaped microtitre plates (Greiner, Nürtingen, F.R.G.) as described (Fleischer and Kreth, 1983a, b). Wells, in triplicate, were filled with 200 μl of complete medium containing 10\textsuperscript{4} target cells and 5 × 10\textsuperscript{4} effector cells. For determination of PHA-dependent cytotoxicity, PHA-P (Wellcome, Grossburgwedel, F.R.G.) was added at a final concentration of 10 μg/ml. Spontaneous release of \textsuperscript{51}Cr was determined by incubating target cells in medium alone or in medium containing PHA. Spontaneous release was usually below 20% of maximal release. Results are expressed as percent specific lysis = (test cpm – spontaneous release) × 100/(maximal release – spontaneous release).

Lymphoproliferative assay

T cell lines (TCLs) were screened for mitogen-induced and antigen-specific proliferative activity by incubating 10\textsuperscript{4} T cells with 5 × 10\textsuperscript{4} irradiated (6000 R) autologous PBL (as a source of antigen-presenting cells) in triplicates of 200 μl in U-shaped 96-well microtitre plates in complete medium. The following antigens were used: rubella antigen (5 hemagglutinating units (HA)/ml), mumps antigen (5 HA/ml), MBP, PLP, galactocerebrosides and actin (50 μg/ml). Control antigen was used at the same dilution as rubella antigen. Cultures were set up for 72 h; 8 h before harvesting, 1 μCi of \textsuperscript{3}H]thymidine (spec. act. 2 Ci/mmol, New England Nuclear, Dreieich, F.R.G.) was added to each well. The cells were harvested with a multiple semiautomated harvester (Skatron) onto glass filter discs and the incorporated radioactivity measured by liquid scintillation counting. Results are expressed as mean cpm of triplicate or, in some instances, duplicate determinations. The standard error of the mean was usually below 10%. A positive response was defined as cpm (antigen)/cpm (medium) > 3.

Results

Generation of TCLs

TCLs were established by seeding CSF cells from frozen samples in the presence of irradiated feeder cells and PHA-containing conditioned medium. The cloning efficiencies were 2% and 4% in separate experiments. All growing colonies from wells that had received 1, 3 or 10 cells and a fraction of growing colonies from 30 cells/well were expanded in lectin-free IL-2. Altogether, 328 TCLs were available for functional analysis. 15 TCLs were derived from one cell/well, 35 TCLs from three cells/well, 60 TCLs from ten cells/well, and 218 TCLs from 30 cells/well. No attempt was made to subclone the TCLs further. TCLs could be maintained in culture for 2–4 weeks without restimulation.

Surface markers

Immunofluorescent staining of uncloned CSF cells showed that 78% were CD4, and 30% CD8 positive. About 50% of the cells expressed HLA-DR antigens. This indicated that a substantial fraction of CSF T cells had been activated in vivo.

Out of 263 TCLs that were stained for surface antigens, 210 (80%) expressed the CD4^+CD8^- and 13 (5%) the CD4^-CD8^+ phenotype. In con-
TABLE 1
CYTOTOXIC ACTIVITY OF T CELL LINES DERIVED FROM CEREBROSPINAL FLUID OF A PATIENT WITH CHRONIC RUBELLA PANENCEPHALITIS

| TCL No. | Percent specific lysis * | Autologous target cells b | Allogeneic target cells b | Percent PHA-dependent cytotoxicity a |
|---------|--------------------------|----------------------------|---------------------------|-------------------------------------|
|         |                          | RV-Infected | RV-Uninfected | RV-Infected | RV-Uninfected | RV-Infected | RV-Uninfected | RV-Infected | RV-Uninfected |
| 2       | 11.7 ± 0.9 d             | < 1         | < 1           | < 1         | < 1           | 8.0 ± 0.7    |
| 41      | 44.7 ± 2.0               | 7.1 ± 0.9   | < 1           | < 1         | < 1           | 20.7 ± 1.0   |
| 44      | 35.0 ± 1.7               | 4.2 ± 0.3   | < 1           | < 1         | < 1           | 28.1 ± 3.3   |
| 75      | 10.4 ± 0.6               | < 1         | < 1           | < 1         | < 1           | 19.2 ± 0.6   |
| 91      | 19.4 ± 1.7               | 1.4 ± 0.2   | < 1           | < 1         | < 1           | 16.4 ± 1.2   |
| 112     | 26.5 ± 2.5               | 2.7 ± 0.1   | 4.0 ± 0.2     | < 1         | < 1           | 20.7 ± 0.7   |
| 133     | 18.8 ± 1.5               | < 1         | < 1           | < 1         | < 1           | 6.6 ± 0.2    |
| 289     | 26.7 ± 7.1               | 10.0 ± 0.3  | < 1           | < 1         | < 1           | 55.4 ± 3.8   |

* Effector to target cell ratio of 2:1 to 5:1.

b HLA types of target cells: Aw23, A26, Bw49, Bw61, Cw2, DR1, DR2 (autologous); A1, B8, B18, DR3, DR5 (allogeneic).
c Rubella virus.
d Mean ± SD.

contrast, 40 T cell lines (15%) contained different proportions of CD4+ and CD8+ cells, indicating lack of clonality. One TCL did not express CD4 or CD8, but CD3 antigens.

**Cytotoxic activity of TCLs**

Screening 191 TCLs for lectin-dependent cytotoxicity against allogeneic uninfected target cells showed that 85 lines (44%) possessed cytolytic activity. Of these TCLs, eight lysed rubella virus-infected target cells. Killing of virus-infected targets was restricted to autologous target cells since no significant lytic activity was observed against rubella-infected allogeneic (HLA-A/B/DR mismatched) PHA-blasts (Table 1). Virus specificity was only tested for two TCLs (TCL 91 and 289), and cytotoxic activity was exclusively directed against rubella-infected and not against mumps-infected target cells (data not shown). Surface marker analysis revealed that all effector cells, except TCL 289, expressed the CD4+ phenotype. All cell lines stopped growing before the precise HLA restriction elements could be identified.

**Rubella-specific proliferation**

A total of 328 TCLs were tested for proliferative reactivity to rubella antigen in the presence of autologous irradiated PBL. Out of these, 26 proliferated in response to rubella antigen, and six lines reacted exclusively to rubella and not to any other viral or non-viral antigen (Table 2). Variability in the magnitude of response may be explained by differences in the activation stage of these cells. All TCLs shown in Table 2 were also tested in cytotoxicity assays, but only TCL 133 was found to exhibit rubella-specific cytotoxicity (Table 1). TCLs 277B, 218 and 212 were stimulated repeatedly with rubella antigen in the presence of autologous irradiated PBL without losing their

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**TABLE 2**

CSF T CELL LINES RESPONDING EXCLUSIVELY TO RUBELLA ANTIGEN

| TCL No. | cpm [3H]Tdr incorporated in response to a |
|---------|-----------------------------------------|
| Medium Rubella | Control Mumps | MBP | PHA |
| 133     | 40 b | 356 ± | 11 c | 66 | 115 | 74 | 1120 |
| H/32    | 60   | 285 ± | 35  | 33 | 58  | 70 | 12036 |
| 277B    | 143  | 3368 ± | 579 | 236 | 217 | 86 | 54069 |
| 212     | 113  | 14628 ± | 1182 | 221 | 397 | 270 | 50190 |
| 218     | 304  | 1248 ± | 122 | 260 | 394 | 195 | 55610 |
| 210     | 120  | 680 ± | 29  | 165 | 70  | 85 | 6180  |

a 10^4 T cells were incubated for 3 days in the presence of 5x10^4 irradiated autologous PBL.
b Means of triplicate or duplicate determinations.
c Means of triplicate determinations ± SD.
**TABLE 3**

| HLA-DR type of antigen-presenting cells | Antigen/mitogen | [³H]TdR incorporated a | TCL 277B | TCL 212 |
|----------------------------------------|-----------------|------------------------|--------|--------|
| DR1,2                                  | Rubella         | 745 ± 102 b            | 4509 ± 374 |
| Control                                | 80 ± 2          | 74 ± 6                 |        |
| DR1                                    | Rubella         | 465 ± 52               | 362 ± 38 |
| Control                                | 84 ± 48         | 316 ± 41               |        |
| DR2                                    | Rubella         | 209 ± 96               | 301 ± 16 |
| Control                                | 179 ± 9         | 169 ± 36               |        |
| DR5,6                                  | Rubella         | 147 ± 42               | 397 ± 20 |
| Control                                | 92 ± 21         | 689 ± 46               |        |
| DR1,2                                  | Mumps           | 183 ± 33               | 373 ± 20 |
| DR1,2                                  | PHA             | 14 124 ± 3057          | 744 ± 65 |

a 10⁴ cells from TCL 277B and TCL 212 were incubated for 3 days with antigens or PHA in the presence of 5 x 10⁴ irradiated autologous and allogeneic HLA-DR-matched or -mismatched PBL. The HLA-DR type of the patient was DR1,2.
b Means of triplicate determinations ± SD.

giving rise to a rubella antigen-specific response. Two TCLs (TCL 277B and 212) were further characterized using rubella antigen together with HLA-DR-matched and -mismatched presenter cells. Table 3 shows that TCL 277B seems to recognize the antigen in the context of HLA-DR1, whereas no restriction element was found in the case of TCL 212 suggesting that class II molecules other than HLA-DR may be the restriction elements for this TCL.

**Response to neuroantigens and to actin**

TCLs were also tested for their proliferative response to neuroantigens (myelin basic protein, proteolipid protein, galactocerebrosides) and to actin. Of 328 TCLs tested, 16 lines demonstrated reactivity to MBP with stimulation indices ranging from 3.5 to 30 times. Of 143 TCLs tested, four lines were specific for PLP and four responded to galactocerebrosides whereas two out of 185 TCLs reacted exclusively to actin. The proliferative responses of 12 representative TCLs are presented in Table 4.

Fourteen out of 16 MBP-reactive TCLs also responded to a minor degree to rubella antigen.

**TABLE 4**

| No. | cpm [³H]TdR incorporated in response to a | Medium | MBP | Galactocerebrosides | PLP | Actin | Rubella antigen | PHA |
|-----|------------------------------------------|--------|-----|---------------------|-----|-------|-----------------|-----|
| 304 | 64 b                                     | 590 ± 16 c | n.t. d | n.t. | 120 | 118 | 11 596          |
| 344 | 107                                      | 576 ± 27 | n.t. | n.t. | 235 | 265 | 3 272           |
| 265 | 96                                       | 1 686 ± 69 | n.t. | n.t. | 728 ± 3 | 450 ± 16 | 12 597          |
| 346 | 35                                       | 1 093 ± 77 | n.t. | n.t. | 308 ± 18 | 1 010 ± 31 | 5 405          |
| 189 | 100                                      | 1 004 ± 53 | n.t. | n.t. | 800 ± 21 | 1 110 ± 31 | 7 637          |
| 1/II| 60                                       | 890 ± 28 | n.t. | n.t. | 220 ± 14 | 476 ± 92 | 7 300          |
| 108 | 125                                      | 10     | 860 ± 15 | 265 | n.t. | 70  | 10 385          |
| 358 | 54                                       | 96     | 2 200 ± 132 | 628 ± 12 | n.t. | 170 | 5 206           |
| 11  | 165                                      | 210    | 192   | 2 490 ± 182 | n.t. | 220 | 1 064           |
| 223 | 150                                      | 365    | 280   | 2 078 ± 51 | n.t. | 380 | 34 106          |
| 80  | 58                                       | 190    | n.t. | n.t. | 675 ± 43 | 154 | 3 295           |
| 356 | 60                                       | 90     | n.t. | n.t. | 540 ± 72 | 142 | 13 580          |

a 10⁴ T cells were incubated for 3 days with and without antigens or PHA in the presence of 5 x 10⁴ irradiated autologous PBL.
b Means of triplicate or duplicate determinations.
c Means of triplicate determinations ± SD.
d Not tested.
Fig. 1. Self-restriction of three antigen-specific T cell lines. T cells were tested for antigen-specific and mitogen-induced proliferative activity in the presence of either autologous (hatched bars) or allogeneic HLA-A/B/DR-mismatched presenter cells (open bars). HLA types were Aw23, A26, Bw49, Bw61, Cw2, DR1, DR2 (autologous) and A1, B8, B18, DR3, DR5 (allogeneic presenter cells). T cell lines were derived from wells originally seeded with one cell (TCL II/29), 30 cells (TCL 314) and ten cells per well (TCL 166).

and/or actin. One of the four PLP-reactive TCLs also recognized galactocerebrosides.

As demonstrated for three TCLs (Fig. 1), antigen-specific proliferation was observed only in the presence of autologous irradiated PBL and not in the presence of HLA-A/B/DR-mismatched presenter cells. No attempt was made to define the restriction specificities of these cell lines.

Discussion

This study describes a patient suffering from a chronic meningoencephalitis. Although rubella virus could not be isolated from CSF or peripheral blood lymphocytes, the serological and immunological findings strongly suggested a persistent infection with rubella virus. This CNS infection was probably established at 8 years of age when the patient was first exposed to rubella virus.

After a latent period of 3 years there was a rapidly progressive deterioration in mental and motor functions. Surprisingly, this was followed by an almost complete clinical remission. Such a disease course is unusual for progressive rubella panencephalitis, not having been described elsewhere in the literature, to our knowledge. However, in subacute sclerosing panencephalitis, another slow virus disease of the CNS, remissions have been described (ter Meulen et al., 1983), extending from a few weeks to several years.

Despite the clinical remission, however, there is strong evidence for a chronic intracerebral inflammatory process in our patient, as indicated by high specific antibody titres in the CSF and a continuing lymphoid pleocytosis.

In the present study, T cells were derived from cryopreserved CSF cells which had been obtained while the patient was in a clinical remission. T cell colonies were set up, without prior in vitro antigenic stimulation, using a standard protocol (Fleischer and Kreth, 1983a, b). We have used this technique recently in acute virus infections of the CNS and have found that analysis of T cell colonies gives reliable information about the specific T cells within the CSF.

Phenotypic and functional analysis of the established TCLs in this case revealed striking differences between acute virus infections of the CNS such as mumps or measles encephalitis, and this chronic disorder. Firstly, the incidence of virus-specific TCLs was low in chronic rubella panencephalitis. Only 26 out of 328 T cell lines responded specifically to rubella virus in either or both of proliferative and cytotoxicity assays. Obviously, the virus-specific cells may be missed easily if only a small sample of the CSF T cell clones is analyzed. This was the reason why we expanded all the T cell colonies, including those found in wells which had originally been seeded with up to 30 CSF lymphocytes (the majority of TCLs tested are therefore oligoclonal cell populations). Secondly, there was a complete lack of
virus-specific CD8$^+$ cytotoxic T lymphocytes (CTL). A small fraction of TCLs did exhibit the typical properties of CTL as far as virus specificity and restriction to self antigens is concerned, but all lines, with the exception of line 289 which lacked both CD8 and CD4 surface markers, were of the CD4$^+$ phenotype. Though not identified sufficiently in this study, the restriction elements are most likely class II HLA molecules.

Class II-restricted CTL clones of the CD4 phenotype have been described in several experimental systems, for example after in vitro restimulation with measles virus (Jacobson et al., 1984) or with herpes simplex virus (Yasukawa and Zarling, 1984). However, the biological significance of these cells in vivo is still a matter of controversy since it cannot be ruled out that all these cells are derived from helper cells which were initially non-cytotoxic but which acquired specific cytotoxic potential during in vitro culture (Fleischer and Wagner, 1986).

We favour the view that class I-restricted cytotoxic T cells of the CD8 phenotype are limited to the initial phase of acute virus infections whereas CD4 cells, whether cytotoxic or not, are a hallmark of a chronic inflammatory process. This view is strengthened by observations in animal models such as Theiler’s murine encephalomyelitis virus infection (Rodriguez et al., 1986) and chronic JHM virus infection in rats (Watanabe et al., 1983) which give rise to chronic inflammatory demyelinating disease. In both conditions the responding T cell is of the helper/inducer type.

In addition to rubella-specific T cells, we were also able to identify T cells with specificity for brain antigens. With regard to neuroantigens we focused on two myelin proteins (MBP, PLP) with potent encephalitogenic properties and on galactocerebrosides which are found on the membranes of oligodendrocytes.

Sensitization to myelin antigens cannot simply be regarded as the result of destruction and release of white matter components because T cells reactive to MBP and PLP have not so far been detected in the CSF of patients with active multiple sclerosis (Fleischer et al., 1984; Hafler et al., 1985). Several mechanisms have been proposed to explain the process of virus-induced autoimmunization, such as the carrier effect of enveloped viruses (Webb and Fazakerley, 1983) or induction of aberrant Ia-antigen expression on tissue cells by either T cell-derived interferons (Bottazzo et al., 1983) or the virus itself (Massa et al., 1986). A third mechanism could involve molecular mimicry between the persisting virus and tissue antigens. For instance, different monoclonal antibodies against measles, herpes and vaccinia virus structural proteins have been shown to react with intermediate filaments (Dales et al., 1983; Fujinami et al., 1983; Sheshberadaran and Norrby, 1984). Moreover, computer-aided analysis had shown sequence homology between peptides of MBP and several viruses (Jahnke et al., 1983).

We are not aware of any published report on shared epitopes between rubella virus and autoantigens. The fact that 14 out of 16 TCLs reacted against MBP, actin and rubella virus can be readily explained by the existence of cross-reacting epitopes, but may also be due to lack of clonality in some of our TCLs. It is, however, statistically unlikely that lack of clonality in the TCLs would account for the observed high frequency of double and treble specificities.

Since rubella virus is a non-cytopathogenic agent it is tempting to speculate on the pathogenic role of myelin-specific T cells. Histopathological examination of brain tissues in PRP has revealed widespread destruction of white matter with loss of myelin, fragmentation of axons, gliosis and scattered perivascular lymphocytes and plasma cells (Townsend et al., 1976). This has been interpreted as a result of a diffuse inflammatory process. It could well be that the extent of tissue destruction is not only due to the presence of immune complexes and/or virus-specific T cells but that destruction of white matter is enhanced by the presence of autosensitized MBP-specific T cells.

These studies document for the first time that virus-specific and autoreactive T cells can be found in the CSF during a chronic virus infection of the CNS in man. Although myelin-specific T cells have not been found in the brain tissue and CSF of patients with MS, a similar mechanism involv-
ing another, unidentified virus and different neuroantigens, such as gangliosides (Bellamy et al., 1986), may play a role in this enigmatic disease.

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