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ISOLATION OF RETROVIRUSES FROM TWO PATIENTS WITH "COMMON VARIABLE" HYPOGAMMAGLOBULINAEMIA

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Summary Retroviruses related to human T-lymphotropic virus III/lymphadenopathy-associated virus (HTLV-III/LAV) have been isolated from peripheral-blood mononuclear cells of two patients with "common variable" hypogammaglobulinaemia who were being treated with intravenous gammaglobulin. One has had three different opportunistic infections. In both patients hypogammaglobulinaemia developed within 6 years of a longlasting undiagnosed viral-like illness in adolescence, and it is suggested that the virus causing that illness also gave rise to the hypogammaglobulinaemia. However, iatrogenic infection from intravenous gammaglobulin cannot be ruled out.

Introduction

"COMMON variable" hypogammaglobulinaemia (CVH) presents at all ages, although the peak incidence is in the third decade.[1] The disease is heterogeneous, although subgroups can be recognised. Most patients have some circulating B lymphocytes, which can be induced in vitro to make IgM and IgG,[2,3] although immunoglobulin production in vivo is impaired. In general these patients are prone to certain bacterial and mycoplasma infections but not to opportunistic viral, protozoal, and fungal infections, such as are seen in patients with severe defects in cellular immunity. Nevertheless, at least 30% of CVH patients have a T-cell lymphopenia and fail to show delayed-hypersensitivity skin reactions.[4,5] In some patients the disease appears to start with a viral-like illness, with malaise, fever, splenomegaly, and lymphadenopathy.

We describe the isolation of retroviruses from two patients with CVH on intravenous gammaglobulin therapy who first presented in adolescence with a viral-like illness. Opportunistic infections characteristic of the acquired immunodeficiency syndrome (AIDS) have developed in one patient; the other remains relatively well.

Case-reports

Table I outlines the initial illness and present clinical status of the two patients. Both patients presented at our clinic with recurrent upper and lower respiratory infections. Neither abused drugs. *Pneumocystis carinii* pneumonia developed in patient 1 4 months after starting intravenous gammaglobulin therapy ('Sandoglobulin', 12 g every 3 weeks). She responded to co-trimoxazole, but 5 months later generalised cutaneous *Herpes zoster* developed, and this responded to acyclovir. She is currently losing weight and has an unexplained fever. She had sexual intercourse for the first time after the development of the *Pneumocystis* infection. Her partner is well and is seronegative for human T-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) by enzyme-linked immunosorbent assay (ELISA) (Burroughs Wellcome). In August, 1983, non-A, non-B hepatitis developed in patient 2, a married man who denied homosexual contact, during a trial of intravenous gammaglobulin.[6] He had previously been on regular intramuscular gammaglobulin therapy for 5 years. His liver function tests returned to normal within a year. He had had about 6 months' treatment with another intravenous preparation ('Gamimune', Cutter) before a retrovirus was isolated from his blood in August, 1984; he has since been receiving regular intravenous 'Sandoglobulin' and remains well apart from episodes of diarrhoea due to chronic salmonella enteritis.

Methods

Immunological Tests (Table II)

Patient 1 was profoundly lymphopenic before treatment, with very low relative numbers of T cells and a reversed T4/T8 ratio. Patient 2 was mildly lymphopenic with a normal percentage of T cells. Lymphocyte transformation with phytohaemagglutinin (PHA), concanavalin A, and irradiated allogeneic cells (mixed

TABLE I—CLINICAL DATA*

Patient	Sex	Hypogammaglobulinaemia diagnosed at (yr)	Present age	Initial illness	Recent clinical status
1	F	19	20	Aged 18 yr—2 months' illness with malaise, cervical lymphadenopathy, and fever	*Pneumocystis carinii* pneumonia August, 1985; severe herpes zoster September, 1985; recurrent bronchitis
2	M	21	33	Hepatitis at 15 yr followed by idiopathic thrombocytopenic purpura and Coombs-positive haemolytic anaemia. Splenomegaly	Mild sinusitis, chronic salmonella enteritis for past year

*Both patients are currently receiving iv gammaglobulin (200 mg/kg/2 weeks).

TABLE II—IMMUNOLOGICAL TESTS

Patient	Lymphocytes			Serum immunoglobulins (g/l)*			Mixed lymphocyte reaction (in vitro)†
	Total/μl	T cells (%)	T4/T8	IgG	IgA	IgM	
1	400–600*	19	0·7	<1	<0·1	1·0	<1‡
2	600–1200	61	2	<1	<0·1	<0·2	31
Normal values	1300–3000	50–82	>1·5	7·2–16·2	0·8–3·9	0·5–3·5	>10

*Before treatment.
†Patient's lymphocytes were mixed with irradiated lymphocytes from a normal subject. Figures given are stimulation indices: $\frac{\text{cpm of patient's + normal cells}}{\text{cpm of patient's cells alone}}$
‡Stimulation indices for PHA and concanavalin A were 2 and <1 respectively.

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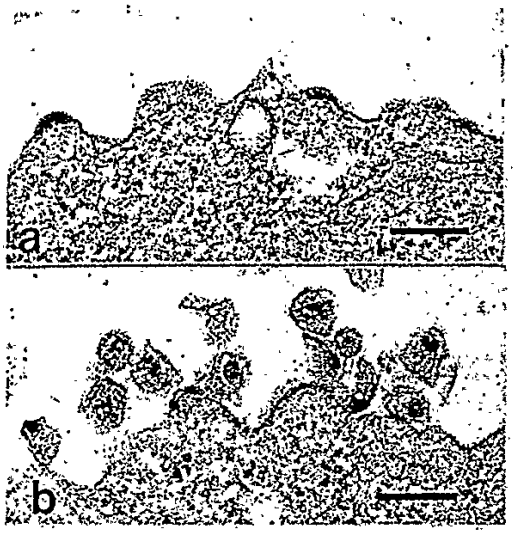

JM cells (CD4+T leukaemia cell line) 1 week after infection with supernatant from co-cultures with lymphocytes from patient 1.

(a) shows several retrovirus particles budding from the plasma membrane; (b) shows released virus particles close to the cell surface. Bar represents 200 nm.

lymphocyte reaction) was severely depressed in patient 1 but within the normal range in patient 2. Both patients' sera were negative for HTLV-III antibodies when tested by means of immunofluorescence against HTLV-III-infected cell lines or by competition ELISA (Burroughs Wellcome).

Virus Isolation Procedures

Peripheral-blood mononuclear cells were separated by 'Ficoll/paque' gradient centrifugation. The cells were grown in RPMI supplemented with 20% fetal calf serum and stimulated with PHA for 24–72 h, after which the cultures were supplemented with recombinant interleukin-2 (IL-2, Biogen). Subsequently, at intervals of 2–3 days, samples from these cultures were co-cultivated with CD4-positive cell lines.[7] Polybrene (4 μg/ml) was added to the co-cultures from patient 1. Reverse transcriptase was measured in the pelleted supernatants from co-cultures.[8]

Syncytium formation was observed in 6 co-cultures from patient 1 in two different laboratories, the first time on cells taken 4 months after the start of intravenous gammaglobulin treatment. Virus isolations were not attempted before treatment. Extensive syncytium formation was seen in polybrene-treated co-cultures, which on electron microscopy showed a retrovirus morphologically indistinguishable from HTLV-III/LAV[9,10] (see figure) and animal lentiviruses. Supernatant from this co-culture was positive for reverse transcriptase, and the cells were positive by immunofluorescence with serum from a patient with AIDS and with the anti-HTLV-III monoclonal antibodies, α-p24 and α-α-p19 (from Dr R. C. Gallo). Southern blots of restricted DNA from infected cells were probed with λBH-10 (from Dr R. C. Gallo). 10 indicated that the viral genome showed homology to HTLV-III/LAV but with restriction enzyme sites distinct from the prototype isolates, HTLV-IIIB and LAV.1. Reverse transcriptase, lentivirus particles, and immunofluorescence with AIDS sera were also found in the co-culture cells from patient 2.

Discussion

There are obvious parallels between the clinical and laboratory abnormalities in some patients with CVH and those with AIDS. Lymphopenia, absent delayed-hypersensitivity skin reactions, and a tendency to autoimmune blood dyscrasias, particularly thrombocytopenic purpura, are common to both disorders.[11,12] At least 10% of CVH patients also have a relative excess of circulating T8 positive lymphocytes.[3,13] The one major laboratory difference between CVH and AIDS is that AIDS patients are usually hypergammaglobulinaemic, although specific antibody production is impaired in many.[14]

Most CVH patients are clinically distinguishable from those with AIDS, since they are not prone to life-threatening opportunistic fungal, viral, and protozoal infections. However, the opportunistic infections seen in AIDS have, rarely, been reported in CVH patients;[15,16] some of these patients have had an associated thymoma.[1] Cutaneous *Herpes zoster* infection is also common in CVH, but it is usually self-limiting and relatively mild.

We looked for retroviruses in CVH patients whose disease had developed in adolescence after a possible prodromal viral-like illness. Retroviruses, morphologically indistinguishable from HTLV-III/LAV, have been isolated from their peripheral-blood mononuclear cells, and severe opportunistic infections characteristic of AIDS have developed in one patient. The viruses were isolated at a time when both patients were receiving intravenous gammaglobulin treatment, and it could be argued that this was the source of infection, particularly in view of a recent report that HTLV-III/LAV may survive the ethanol precipitation used to produce Cohn fraction II material.[17] However, about 30 other CVH patients on regular intravenous gammaglobulin treatment in England have not shown any clinical signs of AIDS, despite receiving gammaglobulin known to contain anti-HTLV-III, and there have been no reports of AIDS in the many hundreds of other patients receiving this treatment throughout the world. Furthermore, patients with hypogammaglobulinaemia who received the same batches of gammaglobulin given to patient 1 have been traced, and none has clinical features of AIDS. Other possible modes of transmission of HTLV-III/LAV need to be considered. Infection through sexual contact is one possibility, particularly since women have contracted AIDS through heterosexual contact.[18] However, neither of our patients was promiscuous. Patient 1 had never had sexual intercourse until after the development of her first opportunistic infection, and her partner is seronegative for HTLV-III. It is possible that hypogammaglobulinaemic patients are particularly prone to HTLV-III/LAV infection through trivial exposure, but there is no evidence that our patients were in contact with anyone considered to be in a high-risk group for AIDS.

The retroviruses isolated from the two CVH patients are clearly related to HTLV-III/LAV, but further analysis is required to establish their identity. It remains to be seen whether this virus is causally related to CVH or is a rare iatrogenic infection.

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differences of below 300 mg/dl. All the patients were enthusiastic. One patient, fearing AIDS contamination of IVIG, discontinued infusions temporarily and was subsequently admitted to hospital with pneumonia; he returned to self-administered twice-weekly infusions. The health of all other patients has been at least as good as that on monthly infusions given in hospital.

Home treatment has saved about $100 per infusion (or $2400–3600 per patient per year). By adjusting time intervals, the dosage can be calculated so that every vial is completely used, thus avoiding waste of IVIG, a further saving.

A further, unexpected benefit was that the instructional sessions provided group support for the process, and a sharing of experiences related to the disease itself bolstered coping skills. The self-administration of IVIG, in the comfort of their home, reduced inconvenience and time lost from work or school, and also provided greater independence and more personal control of disease.

We conclude that the home administration of IVIG is both feasible and safe. It permits more frequent infusion and the maintenance of near-normal serum IgG levels. Home administration is more convenient and cheaper than hospital treatment, and the patients are pleased with the change.

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1. Mease PJ, Ochs HD, Wedgwood RJ. Successful treatment of echovirus meningoencephalitis and myositis-fasciitis with intravenous immune globulin therapy in a patient with X-linked agammaglobulinaemia. *N Engl J Med* 1983; 308: 1278–81.
2. Morell A, Skvaril M, Barandun S. Build-up and maintenance of IgG serum concentrations with intravenous immunoglobulin in patients with primary humoral immunodeficiency. *Vox Sang* 1982; 43: 212.
3. Ochs HD, Fischer SH, Wedgwood RJ, et al. Comparison of high-dose and low-dose intravenous immunoglobulin therapy in patients with primary immunodeficiency diseases. In: Proceedings of Symposium on Intravenous Immune Globulin and the Compromised Host. *Am J Med* 1984; 76(3A): 78–82.

GUT TRANSFER OF ENVIRONMENTAL PLUTONIUM AND AMERICIUM

SIR,—Dr Hunt and his colleagues (Feb 22, p 439) have painstakingly assessed the transfer of environmental plutonium and americium across the human gut. However, their view—that their results suggest that currently accepted gut transfer factors may be excessively "conservative"—is open to question. Only two measurements of the absorption of plutonium from the human gastrointestinal tract have been reported—one by Hunt and colleagues for absorption from "Sellafield shellfish" and the other by Mussalo-Rauhamaa et al[1] for absorption from reindeer liver contaminated by fall-out plutonium. Both groups are careful to point out that the problems of analysis and the assumptions made concerning the intake and excretion introduce considerable uncertainty into their calculations; further, neither reindeer liver nor Sellafield shellfish are typical components of the diet. Thus, very valuable though they are, these human data must be treated with some caution in the assignment of numerical values for gut transfer factors.

A very large body of animal data on the absorption of plutonium from the gastrointestinal tract of various species has shown that absorption may be influenced by several factors including mass ingested, dietary status, presence of complexing anions, and, perhaps, disease states or drugs. All these factors must be taken into account in proposing a value for the gut transfer factor. Because of the wide variations in the data reported and the difficulties in accurately measuring very low levels of absorption it has been argued, in a report submitted to the International Commission on Radiological Protection,[2] that the data do not permit a gut transfer factor (f_1) for plutonium and the trivalent actinides to be specified more precisely than a whole order of magnitude. Since the reported data suggest a value for f_1 larger than 10^{-4} and less than 10^{-3}, it was concluded that a value of 10^{-3} would give a sufficient margin of safety for radiation protection purposes in all situations where the intake cannot be described precisely. The human data ($8-9\times10^{-4}$ [Mussalo-Rauhamaa et al] and $0{\cdot}8\pm0{\cdot}3\times10^{-4}$ [Hunt et al]) give added confidence to the proposed range and also suggest that the proposed upper value of 10^{-3} would not be excessively cautious. There may be situations in which this cautious value for f_1 of 10^{-3} would need to be replaced by a different value more appropriate to the specific situation.

In view of public concern about the effects of a possible release of radionuclides into the environment it is important for those of us involved in radiation protection to be open in our discussion of the uncertainties in the biological and other indices that we use. We should be careful to avoid giving, even unintentionally, an unjustified impression of precision.

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1. Mussalo-Rauhamaa H, Jaakola T, Miettinen JK, Laiho K. Plutonium in Finnish Lapps: an estimate of the gastrointestinal absorption of plutonium by man based on a comparison of the plutonium content of Lapps and Southern Finns. *Hlth Phys* 1984; 46: 549–59.
2. International Commission on Radiological Protection. The metabolism of plutonium and related elements: Report of an ICRP Task Group, 1986. (Submitted for adoption and subsequent publication in *Ann ICRP.*)

HTLV-III SEROCONVERSION ASSOCIATED WITH HEAT-TREATED FACTOR VIII CONCENTRATE

SIR,—Patients with haemophilia are at high risk of HTLV-III infection and AIDS from contaminated clotting factor concentrates. Although HTLV-III has not been demonstrated in these concentrates, the prevalence of HTLV-III antibody in haemophilic patients is high. On the basis of the known sensitivity of HTLV-III to heat and of studies demonstrating inactivation of HTLV-III in spiked concentrates,[1,2] the Centers for Disease Control and the Medical and Scientific Advisory Council of the National Hemophilia Foundation in the United States have recommended the preferential use of heat-treated factor VIII preparations over cryoprecipitate, plasma, or non-heat-treated materials. Preliminary studies have revealed an absence of HTLV-III seroconversion in previously untreated patients receiving heat-treated products.[3–5] However, these studies have been done on small numbers of patients; some have had mild or moderate disease and their infrequent treatment may not have resulted in a sufficiently heavy inoculum of surviving virus to induce seroconversion. We report here a case of HTLV-III seroconversion in a mild haemophiliac after administration of high doses of heat-treated factor VIII concentrate.

A 31-year-old man with mild haemophilia A was transferred to the emergency room at North Carolina Memorial Hospital on June 30, 1985, with a large haematoma in his left leg and an acute compartment syndrome. He had been treated with cryoprecipitate in his youth but had not had plasma, cryoprecipitate, or other blood products since 1975. He admitted to previous drug abuse, including intravenous drugs, but denied use of intravenous drugs in the previous 7 years. He had no other risk factors for AIDS.

An emergency four-compartment fasciotomy was done after administration of heat-treated factor VIII concentrate. Packed red blood cells from two female donors who were HTLV-III negative by ELISA were administered during the operation. Over the 20 day convalescent period, the patient received 99 960 units of heat-treated concentrate from five different lots obtained from a single manufacturer. Each lot was confirmed to have been heat-treated by the manufacturer.

On July 24, 25 days postoperatively, the patient experienced a febrile illness characterised by headache, chills, malaise, photophobia, generalised myalgia, and fever (38·7°C). Physical examination then was significant for new, bilateral, tender cervical lymphadenopathy, tonsillar enlargement without exudate, and an erythematous rash over the back. There was no hepatosplenomegaly. Complement fixation titres for toxoplasma were less than

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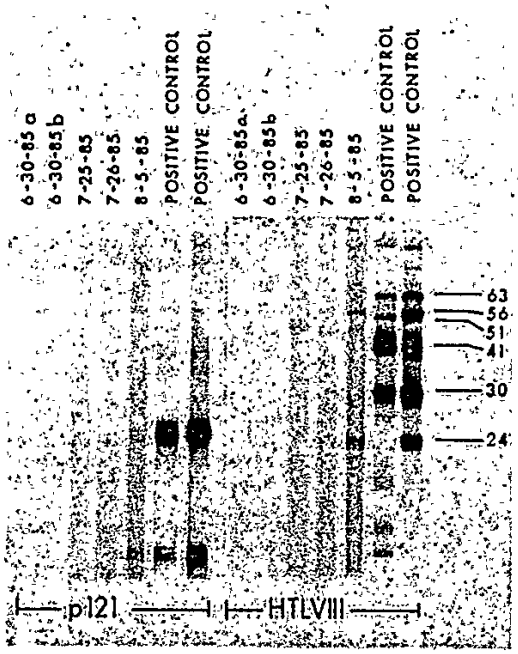

HTLV-III reactivity by western blot analysis.

HTLV-III directed antibody was detected using SDS-disrupted HTLV-III (right panel) and recombinant peptide 121 (left panel) as antigen. Sera from successive bleeds were tested at a 1:50 final dilution and binding antibody was localised with ^{125}I-staphylococcal protein A. Sample 6–30–85a was obtained on admission before treatment with factor VIII; 6–30–85b, from day of admission after treatment with factor VIII; 7–25–85, from day of admission with febrile illness; 7–26–85, second sample from admission with febrile illness; 8–5–85, from follow-up clinic visit. Molecular weights of viral polypeptides are on right. Recombinant peptide 121 was a gift from Centocor Laboratories.

16. Serum titres against Epstein-Barr viral capsid and early antigen were suggestive of recent exposure. Antibody titres (inverse) were: IgG <1640, capsid IgM 10, early antigen more than 20, nuclear antigen 5. A 'Monospot' test was negative. Liver function tests were normal and he was hepatitis antigen negative. Anti-HBs was positive. The patient recovered fully with supportive therapy.

ELISA for HTLV-III antibody was not done. All HTLV-III antibody studies were done by western blot. The figure shows a western blot analysis of serum samples obtained at various times during the patient's postoperative course. The sample labelled "6–30–85a" was taken before factor VIII infusion and that labelled "6–30–85b" was taken several hours after infusion. Samples were tested on nitrocellulose strips containing SDS-disrupted HTLV-III as well as recombinant peptide 121 (p121). p121 contains about one-half the aminoacid sequence of the viral transmembrane protein gp41 and has been shown to be reactive with most HTLV-III antibody positive sera.[6] Specific antibody was only apparent in the last blood sample (Aug 8) and this sample showed reactivity to both the recombinant envelope peptide 121 as well as to the internal core antigens of HTLV-III (p24) and the uncleaved precursor of the internal antigen (p56). Lack of sensitivity to gp41 on the strips containing disrupted HTLV-III despite binding to the analogous sequences in the recombinant peptide 121 is not unusual. This is thought to be related to increased detection sensitivity of these antibodies when higher levels of the target antigens are loaded on the test strips. This pattern of reactivity is also characteristic of sera taken relatively early during the infectious process.

While the seroconversion encountered in this case appears to represent a specific immune response against HTLV-III antigens, the significance of this response is uncertain. Although a single attempt to detect live virus in the patient's lymphocytes at the time he was seropositive was unsuccessful, the possibility remains that in this case, heat treatment failed to inactivate virus and immunity was in response to infectious virus. The finding of an inverted T4:T8 ratio (0·62) at the time seroconversion was first noted suggests live virus transmission. Also, the febrile illness 25 days postoperatively could have been acute HTLV-III infection.[7] Another possibility is that inactive virus or viral fragments provoked the immune response. If viral material is present in concentrates, heat treatment might inactivate virus, but viral antigen might still be present in amounts sufficient to elicit an immune response. Seroconversion in this case would not signify exposure to active virus. Another possibility is passive transfer of HTLV-III antibody. IgG is present in factor VIII concentrates but we have been unable to identify antibodies against HTLV-III proteins in the concentrates we have tested and one would have expected the samples of June 30 and July 25 to have been antibody positive. A final possibility is that the patient acquired HTLV-III through intravenous drug use, despite his denial of current activity. More recent specimens are not available; we have lost touch with this patient.

This case illustrates the need for further studies of heat-treated factor VIII and factor IX concentrates with the aim of determining as rapidly as possible how frequently HTLV-III seroconversion occurs after administration of heat-treated product and whether seroconversion reflects a response to live or attenuated virus. The urgency of these studies is obvious in view of the need to make reliable recommendations for safe treatment of haemophilic patients.

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1. McDougal JS, Martin LS, Cort SP, Mozen M, Heldebrant CM, Evatt BL. Thermal inactivation of the acquired immunodeficiency syndrome virus, human T lymphotropic virus-III/lymphadenopathy-associated virus, with special reference to antihemophilia factor. *J Clin Invest* 1985; 76: 875–77.
2. Piszkiewicz D, Klagsbrun HS, Apfelzweig R, et al. Inactivation of HTLV-III/LAV during plasma fractionation. *Lancet* 1985; ii: 1188–89.
3. Rouzioux C, Chamaret S, Montagnier L, Carnelli V, Rolland G, Mannucci PM. Absence of antibodies to AIDS virus in haemophiliacs treated with heat-treated factor VIII concentrate. *Lancet* 1985; i: 271–72.
4. Mosseler J, Schimpf K, Auerswald G, Bayer H, Schneider J, Hunsmann G. Inability of pasteurised factor VIII preparations to induce antibodies to HTLV-III after long-term therapy. *Lancet* 1985; i: 1111.
5. Felding P, Nilsson IM, Hansson BG, Biberfeld G. Absence of antibodies to LAV/HTLV-III in haemophiliacs treated with heat-treated factor VIII concentrate of American origin. *Lancet* 1985; ii: 832–33.
6. Chang TW, Kato I, McKinney S, et al. Detection of antibodies to human T-cell lymphotropic virus-III (HTLV-III) with an immunoassay employing a recombinant *Escherichia coli*-derived viral antigenic peptide. *Biotechnology* 1985; 3: 905–09.
7. Ho DD, Sarngadharan MG, Resnick L, Dinarzo-Veronese F, Rota TR, Hirsch MS. Primary human T-lymphotropic virus type III infection. *Ann Intern Med* 1985; 103: 880–83.

RATIONALE FOR EARLY USE OF LEVODOPA IN PARKINSONISM

SIR,—It has been suggested that levodopa may cause permanent changes in the nervous system that give rise to fluctuations in clinical response.[1] If so, it would seem sensible to withhold this drug for as long as possible and to treat early parkinsonism with other medications.

There is considerable evidence that it is the loss of dopaminergic neurons and not levodopa therapy that is the cause of fluctuations:

(1) Fluctuations are more commonly seen in advanced, severe cases of this degenerative disease.[2,3]

(2) Fluctuations are encountered early in the treatment of acute, severe parkinsonism in young people exposed to 1-methyl-4-