Macaque Monkey Type D Retrovirus Replicates in vitro in a Distinct Subpopulation of B Lymphocytes

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SUMMARY

Type D retroviruses have recently been shown to induce a wasting syndrome with associated lymphadenopathy, thymic atrophy and transient decreased peripheral blood lymphocyte blastogenic responsiveness in juvenile macaque monkeys. The replication in vitro of D/New England virus was assessed in various lymphocyte subpopulations to determine the possible pathogenesis of the immune dysfunction induced by this virus. While D/New England did not replicate in cultured T lymphocytes or induce any demonstrable dysfunction of T cells in vitro, it did grow in the cells of the B lymphocyte lineage. D/New England growth occurred in vitro in African Burkitt's lymphoma and pre-B cell lines, but not in Epstein-Barr virus-transformed normal B lymphocytes. The infection of a restricted B lymphocyte population by this primate type D retrovirus may play a role in the aetiology of the immune abnormalities which it induces.

Type D retroviruses have recently been recognized as common, horizontally transmitted infectious agents in macaque monkeys. The first isolate of such a virus, the Mason-Pfizer monkey virus, was described in 1970 (Chopra & Mason, 1970). Related viruses have been recently isolated at the New England (D/New England) (Daniel et al., 1984; Desrosiers et al., 1985), California (SAIDS retrovirus) (Marx et al., 1984), Washington (D/Washington) (Stromberg et al., 1984) and Oregon (Marx et al., 1985) Regional Primate Centers. Their isolation from monkeys which had died with a wasting syndrome with associated immunological abnormalities led to their initial implication as the causative agent of an immunodeficiency syndrome of macaques. Subsequent experimental inoculation of healthy monkeys with these isolates has demonstrated that these viral isolates do induce a sometimes fatal wasting syndrome with associated lymphadenopathy, thymic atrophy and transient decreased peripheral blood lymphocyte (PBL) blastogenic responsiveness (Fine et al., 1975; Letvin et al., 1984; Desrosiers et al., 1985; Marx et al., 1984). The mechanism by which viruses of this group induce immune dysfunction is not known. The present studies were initiated to explore this issue.

The tropism of some viruses for cells of the immune system has been shown to account, at least in part, for the immune dysfunction induced by these agents (Klatzmann et al., 1984; Dalgleish et al., 1984; Kannagi et al., 1985). Therefore, type D retrovirus replication in T cell cultures and the effect of the presence of that virus on T cell growth and function were assessed. A stock of D/New England was prepared on Raji cells. Human and Macaca mulatta PBL were stimulated in bulk culture with phytohaemagglutinin; 2 days later the cells were washed and resuspended in flasks at a cell concentration of \(7 \times 10^5\)/ml in crude human interleukin-2 (IL-2)-containing medium and 10% D/New England-containing supernatant [3-6 \times 10^4\) c.p.m. reverse transcriptase (RT) activity/ml, an m.o.i. of 2]. Every 3 to 4 days culture supernatants were assessed for RT activity as an indication of virus replication and viable cells were counted. While RT activity was generated in a parallel culture of similarly infected Raji cells, none was detected in the supernatants of the monkey or human T cell cultures. We could not rule out the possibility that culture supernatants in which no RT activity was detected might still contain small
quantities of viable virus. Therefore, rhesus monkey T cell lines maintained in human IL-2 were incubated with culture supernatants containing virus (10⁶ infectious particles/ml) for 2 h, washed and maintained in culture for 7 days thereafter. At the end of this time the culture supernatants were assessed for their ability to induce syncytium formation in Raji cultures and these T cells were examined by electron microscopy. No syncytia were seen in these Raji cultures and examination of the T cell lines by electron microscopy revealed no evidence of virus. These experiments suggested that no viral growth occurred in primate T lymphocytes. Furthermore, T lymphocyte numbers in cultures maintained with and without virus were equivalent as long as 28 days after initiating such cocultivations, indicating that D/New England exerted no toxic effect on these T cells.

The effect of this type D retrovirus on macaque monkey T lymphocyte function was assessed by adding infectious viral particles to PBL in vitro and determining the proliferative capacity of these cells. There was no alteration of the proliferative response of the M. mulatta or M. cyclopis PBL to concanavalin A, pokeweed mitogen or xenogeneic stimulator cells. Finally, T lymphocyte lines derived from three macaque species, M. mulatta, M. fascicularis and M. cyclopis were grown in vitro in the presence of this virus and assessed frequently for an acquired capacity to grow autonomously without IL-2, an indication of transformation. No such autonomous growth was observed.

D/New England replication was also assessed in cells of the B lymphocyte lineage. Interestingly, viral growth was detected in the cell lines BJAB, Raji, Ramos and Daudi, all the African Burkitt's lymphoma cell lines tested (Fig. 1 a). D/New England growth also occurred in the pre-B cell lines PB 697 and Nalm 6 (Fig. 1 b). However, no RT activity was generated in cultures of virus with human, common marmoset (Callithrix jacchus) or owl monkey (Aotus trivirgatus) B lymphocytes which had been transformed in vitro with Epstein–Barr virus (EBV) (Fig. 1 c).

African Burkitt’s and pre-B cell lines have been shown clearly to differ from B cells transformed in vitro by EBV in their state of activation and the surface antigens which they
played a role in the differential susceptibility of these B lymphocyte populations to infection by D/New England. Human sheep erythrocyte rosette receptor-negative PBL, a B lymphocyte-enriched cell population, were stimulated in vitro with a series of B cell lectins, including pokeweed mitogen, staphylococcal protein A and EBV, and then cocultivated with cell-free D/New England-containing culture supernatants. Supernatants from these cocultivations sampled every 3 to 4 days for a 3 week period never contained detectable RT activity. This suggested that the state of activation by itself may not be the critical factor in determining B lymphocyte susceptibility to type D retrovirus growth.

Some viruses have been shown to infect subpopulations of lymphocytes through binding to a specific membrane structure expressed on a restricted lymphocyte subpopulation (Klatzmann et al., 1984; Dalgleish et al., 1984; Kannagi et al., 1985; Fingeroth et al., 1984). We therefore attempted to determine whether an antigenic structure expressed by cells of the Burkitt's lymphoma lines but not by the EBV-transformed B cells plays a role in the infection of B lymphocytes by type D retroviruses. Growth of D/New England on the type D retrovirus-permissive cell line Ramos could not, however, be blocked in vitro by the addition of five different monoclonal antibodies (anti-HB8, anti-HB9, anti-HB10-11, anti-B2 and anti-B4) (Calvert et al., 1984; Tedder et al., 1985; Nadler et al., 1981, 1983), each of which recognizes a structure expressed on the Burkitt's lymphoma-derived cells but not the EBV-transformed normal B cells. Thus, while another structure expressed on the African Burkitt's lymphoma-derived cells but not the EBV-transformed normal B cells may be the receptor for the type D retrovirus, we were unable to define that structure.

Although the type D retrovirus infects a restricted population of lymphocytes in vitro, the mechanism by which the selectivity occurs remains unclear. This phenomenon of a selective infection of B lymphocytes may play a role in the aetiology of the immune abnormalities induced by the type D retrovirus in non-human primates.

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REFERENCES


Short communication


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