Different Roles for L3T4+ and Lyt 2+ T Cell Subsets in the Control of an Acute Herpes Simplex Virus Infection of the Skin and Nervous System

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SUMMARY

Rat monoclonal antibodies were used to deplete selectively Lyt 2 (cytotoxic) and L3T4 (helper) T cell populations in vivo. These antibodies produced > 95% depletion of the respective T cell subset as determined by fluorescent antibody and cytofluorographic analyses. Antibody-treated mice were infected in the ear pinna with herpes simplex virus (HSV) and the induction of virus-specific T cell and antibody responses were monitored during the acute infection. Lyt 2-deficient mice produced delayed hypersensitivity and HSV-specific antibodies comparable to those in untreated animals. However, major histocompatibility complex class I-restricted T cell killing was abolished. In contrast, L3T4-deficient animals failed to produce either primary delayed hypersensitivity response or specific antibodies to the virus, but cytotoxic T cell responses were induced and even augmented in comparison with infected, normal animals. This observation clearly demonstrates that Lyt 2 cytotoxic T cells can be induced in a helper T cell-deficient environment. The ability of T cell subset-deficient mice to clear infectious virus was investigated in the skin of the ear and the part of the nervous system innervating the site of infection. L3T4-deficient animals showed a markedly delayed clearance of virus from the ear and also had a more florid infection of the nervous system. However, Lyt 2-deficient mice cleared the infection in the ear normally, but a severe infection of the nervous system was still observed. The implication of these observations to the pathogenesis of this virus is discussed.

INTRODUCTION

Immunosuppression has been widely used to study the nature of the immune response in infectious diseases. In particular, deficiencies in T lymphocyte function often predispose the mammalian host to a more severe acute viral infection. This is clearly demonstrated in athymic nude mice infected percutaneously with herpes simplex virus (HSV), which show a progressive infection of the nervous system resulting in the death of the animal (Kapoor et al., 1982). Attempts to identify the role of particular T cell populations in protection have relied upon the adoptive transfer of immune cells to infected recipients and correlating the functional activity of these cells with the clearance of infective virus (Nash et al., 1985). Other, less-selective, procedures for inducing immunosuppression in vivo have involved the interference with T cell (and B cell) functions using the cytostatic drugs cyclophosphamide and cyclosporin A.

Recently, Cobbold et al. (1984) used rat IgG2b monoclonal antibodies against the L3T4 or Lyt 2 membrane antigens to deplete the appropriate T cell subpopulation in vivo. The depletion observed was > 95%, and led to diminution in the functional properties attributed to these T cell populations. Furthermore, when adult thymectomized animals were used, the depletion was long-lived, with no apparent recovery of the depleted subsets. This approach was used to study the role of T cells in the rejection of skin grafts (Cobbold et al., 1984; Cobbold & Waldmann, 1986), and recently by Benjamin & Waldmann (1986) to induce tolerance to protein antigens in
animals depleted of L3T4 cells. Clearly, the application of these monoclonal antibodies has far-reaching implications for studies on T cell immunology in general, and in particular for investigations on the role of T cells in immunity to viruses, and also on the immunopathology of viral diseases.

In this paper, the antibodies described by Cobbold et al. (1984) were used to deplete selectively L3T4 and Lyt 2 T cell subsets, in order to study the role played by these T cells in controlling an acute HSV infection (Hill et al., 1975).

In particular, we addressed the following questions on the behaviour of T cells during a viral infection. Can Lyt 2 (cytotoxic) T cells be induced in a helper T cell-deficient environment? Are there distinct T cell subsets checking infections in different host tissues, e.g. skin and nervous system? Is there redundancy in the T cell response of mice to an HSV infection?

The results clearly indicate that virus-specific cytotoxic (Lyt 2-type) T cells can be induced in L3T4-deficient mice, and that these cells function in protecting the nervous system from infection by the virus, but that they are not a major effector cell in an acute herpesvirus infection of the skin. In the latter tissue, L3T4 cells act as the major effector T cell population, possibly by mediating a delayed-type hypersensitivity response.

METHODS

Mice. Female CBA mice were obtained from the Department of Pathology Animal House and used when 6 to 8 weeks old.

In some experiments the mice were thymectomized at 6 weeks by standard surgical procedures, under hypnorm/valium anaesthesia. These mice were used in experiments 2 weeks later.

Virus. HSV type 1, strains SC16 and CI(101)TK? were used. The origins and pathogenicity of these viruses for mice have been reported elsewhere (Field & Wildy, 1978). Stocks were grown in BHK-21 cells and stored at -70 °C.

Antibodies. Rat IgG2b monoclonal antibodies with specificity for the following antigens were used: L3T4 (YTS 191.1) and Lyt 2 (YTS 169.4). These antibodies were partially purified from ascitic fluid by precipitation with 50% (NH₄)₂SO₄. The antibodies were dialysed against phosphate-buffered saline and stored at -20 °C at a concentration of 5 to 10 mg protein/ml (equivalent to 1 to 2 mg/ml of active antibody). Details on the derivation, specificity and activity of these antibodies in vivo have been described elsewhere (Cobbold et al., 1984).

To detect the rat monoclonal antibodies on T cells, a fluorescein isothiocyanate (FITC) mouse anti-rat light chain (FITC-MAR 18.5) monoclonal antibody was used (Lanier et al., 1982).

Depletion of T cells in vivo. Mice were injected intravenously with 0.1 ml of stock antibody preparations, i.e. 100 to 200 μg specific antibody/injection. The antibody was given twice 2 days before and 2 days after infesting the animals with the virus. Thereafter, the mice were maintained on a standard diet and water.

Infection of mice and assay for virus infectivity. SC16 (10⁴ to 10⁵ p.f.u.) was injected into the left ear pinna of anaesthetized mice, as previously described (Nash et al., 1980a). At intervals thereafter, tissues were removed (i.e. ear pinna, C2, C3, C4 cervical ganglia and spinal cord adjacent to the ganglia) and the amount of virus was determined using a standard plaque assay in BHK-21 cells (Nash et al., 1980a).

Measurement of the primary delayed-type hypersensitivity response. Details of the procedure used to measure the inflammatory response and delayed hypersensitivity have been described elsewhere (Nash et al., 1980b). The infiltration of cells into the infected ear was measured on successive days using a micrometer screw gauge. The results were expressed as the difference in thickness of the infected versus the uninfected ear and expressed as units × 10⁻² mm.

Detection of virus-specific antibodies. The method used was based on that described by Colombatti & Hilgers (1979). Briefly, sera taken from mice at different times after infection were added at various dilutions to SCI6-infected BHK cell monolayers fixed with glutaraldehyde. The bound antibody was detected using ¹²⁵I-labelled Protein A (Amersham). The results were expressed as the serum dilution giving counts 2 standard deviations above background, i.e. counts produced by normal mouse serum.

Assay for cytotoxic T cell responses. The assay used was that described by Nash et al. (1980b), with some modification. Briefly, draining lymph nodes were removed from mice 7 days after infection of 10⁴ p.f.u. SC16 into the ear. Two × 10⁶ lymph node cells were cultured for 4 days with 2 × 10⁶ X-irradiated syngeneic spleen cells, infected with SC16 (m.o.i. 5). The cultured cells were then mixed with suspension L929 cells labelled with ⁵¹Cr (sodium chromate, 100 μCi/10⁶ cells; Amersham) and either infected with SC16 (m.o.i. 10) or left uninfected. After incubating for 5 h at 37 °C the release of ⁵¹Cr into the cell supernatant was measured using an LKB gamma counter. The percentage specific lysis was determined as follows: (experimental group - spontaneous release/maximum release - spontaneous release) × 100.
Use of flow cytometry to analyse fluorescent staining cells. Lymph node cells were obtained at various times after infection and stained for Lyt 2 and L3T4 antigens. The technique was that described by Cobbold et al. (1984). The lymph node cells were incubated with 100 µg/ml of YTS 169.4 (anti-Lyt 2) or YTS 191.1 (anti-L3T4) containing 0.1% NaN₃ and 10% heat-inactivated (56 °C for 1 h) rabbit serum for 1 h at 4 °C. Unbound antibody was washed away and a 20 µg/ml FITC-labelled mouse anti-rat light chain (MAR 18.5) was added for a further 1 h at 4 °C. The cells were washed in medium containing 50 µg/ml propidium iodide (to stain dead cells) and resuspended in phenol-free medium containing 0.1% NaN₃. The samples were then analysed using an Ortho H-50 cytofluorograph linked to a 2150 4-parameter computer (Ortho Diagnostic Systems Ltd, High Wycombe, U.K.) at a flow rate of 1000 cells/s. Computer gating was used on forward and 90° light scatter and on red fluorescence (propidium iodide) such that histograms of green (fluorescein) fluorescence were obtained for live single cells.

RESULTS

Membrane phenotype of lymph node cells taken from antibody-treated mice

A group of CBA mice were injected intravenously with either YTS 191.1 (anti-L3T4) or YTS 169.4 (anti-Lyt 2), 2 days before and 2 days after virus infection. On day 7 the lymph node draining the site of infection was removed and the cells stained for either the presence of Lyt 2 or L3T4 antigens. The percentage of cells expressing these antigens together with the relative fluorescent intensity of the antigen on each cell is shown in Fig. 1. Following the injection of anti-L3T4, only 1-2% weakly fluorescent L3T4 cells were detected, whereas the percentage of Lyt 2 cells increased to 60%. Conversely, following the injection of anti-Lyt 2, only 1-2% weakly staining Lyt 2 cells remained. Similar results have been obtained with cell suspensions taken from the spleens of antibody-treated animals (data not shown).

As reported by Cobbold et al. (1984), antibodies injected into adult thymectomized mice led to a depletion of T cells which was long-lasting. The number of Lyt 2 cells in anti-Lyt 2-treated adult thymectomized CBA mice was monitored over a long time period. As early as 4 days after and up to at least 180 days the number of Lyt 2 cells in the draining lymph node of depleted mice was 1-2 to 1-8% and in the undepleted controls was 16 to 23%.

Another interesting feature of this system is that relatively small amounts of anti-Lyt 2 (1 to 2 µg/injection) were required to achieve >95% depletion of Lyt 2 cells, when compared with anti-L3T4, where 100 to 200 µg were required to give >95% depletion.

Effect of T cell depletion on the induction of delayed hypersensitivity

CBA mice were injected with either anti-L3T4, anti-Lyt 2 or a combination of both. Each group was injected with 10⁵ p.f.u. SC16 and the ear thickness was measured at 24 h and on successive days. As shown in Fig. 2, depletion of Lyt 2 cells did not affect the swelling reaction, whereas depletion of L3T4 cells, or of both L3T4 and Lyt 2 cells reduced the cellular infiltration from day 5 onwards.

Antibody response to the virus in depleted mice

Mice injected with anti-L3T4, anti-Lyt 2 or a combination of both were then infected in the ear with 10⁴ p.f.u. SC16 and at time intervals thereafter were bled to determine the antibody response to the virus. As shown in Fig. 3, depletion of Lyt 2 cells did not affect the titre of the virus-specific antibody, compared to sera from untreated animals. Removal of L3T4 cells or all T cells abrogated the antibody response. These data support previous observations on the thymus dependence of the antibody response to HSV (Kapoor et al., 1982; Burns et al., 1975).

Induction of cytotoxic T cells in subset-depleted mice

To investigate the cytotoxic T cell response in subset-depleted mice, draining lymph node cells were obtained 7 days after injection of 10⁴ p.f.u. SC16 in to the ear pinna. The lymph node cells were restimulated with virus in vitro and held for 4 days and then mixed at various effector:target cell ratios with ⁵¹Cr-labelled virus-infected or uninfected L cells (H-2k). The results of two separate experiments are shown in Fig. 4. T cell killing is invariably low in this virus-host system. (Lymph node cells from animals not treated with antibody showed between 9 and 16% specific killing at 40:1 over the two experiments.) Nevertheless, the depletion of Lyt 2
cells reduced the killing to background levels. In contrast to the above results, depletion of L3T4 cells produced a markedly enhanced killing over the controls which were not treated with antibody. This result was unexpected in view of the importance of L3T4 cells in the induction \textit{in vitro} of allo-specific cytotoxic T lymphocyte responses (Cobbold \textit{et al.}, 1984; Cantor & Boyse, 1975). Furthermore, once the cytotoxic cells had become activated in these 'helperless' mice, remixing L3T4-depleted with Lyt 2-depleted lymph node cells could not reduce the cytotoxic response to the levels seen in untreated mice (Fig. 4b).

The killing observed in these experiments was major histocompatibility complex (MHC)-restricted, i.e. no killing of H-2\textsuperscript{d} 3T12 fibroblast cells was observed and was virus-specific (data not shown; Nash \textit{et al.}, 1980).
HSV infection in T cell subset-deficient mice

Fig. 3. Herpesvirus-specific antibody production in antibody-treated or untreated mice. All mice were injected in the ear pinna with $10^4$ p.f.u. SC16 and the specific antibody response was determined for mice treated with YTS 191.1 (a), YTS 169.4 (b) or untreated (c). The mean antibody titre is shown for five mice/group; deviation from the mean was 15% or less.

Fig. 4. Induction of cytotoxic T cell responses in antibody-treated or untreated mice injected in the ear pinna with $10^4$ p.f.u. SC16. Two experiments are shown comparing the specific cytotoxic reaction shown in YTS 191.1-treated (a), YTS 169.4-treated (b) and untreated (c) mice. In (b) the cells from YTS 191.1-treated and YTS 169.4-treated mice were mixed at the start of the 5 day culture period (see Methods) and specific killing was determined (c). Each point represents the mean of triplicate cultures of cells pooled from the lymph nodes of three mice; deviation from the mean was always less than 10%.
Fig. 5. Yield of HSV in the ears of normal or T cell-deficient CBA mice. Each mouse received 10^4 p.f.u. SC16 in the left ear pinna following treatment with YTS 191.1 (△), YTS 169.4 (■), YTS 191.1 + YTS 169.4 (●) or no antibody (○). Each point represents the mean and range log_{10} p.f.u. of five mice/group. Arrows indicate values less than 1 log_{10} p.f.u.

Fig. 6. Yield of HSV in (a) the spinal cord and (b) the dorsal root ganglia of T cell-deficient or normal mice. Each mouse received 10^4 p.f.u. SC16 in the left ear pinna following treatment with YTS 191.1 (△), YTS 169.4 (■), YTS 191.1 + YTS 169.4 (●) or no antibody (○). Each point represents the mean and range log_{10} p.f.u. of five mice/group. Arrows indicate values less than 1 log_{10} p.f.u.

Effect of T cell depletion on the spread of the virus

CBA mice depleted of L3T4, Lyt 2 or L3T4 + Lyt 2 cells were infected in the ear pinna with 10^4 p.f.u. SC16. At various times thereafter, the ear, ganglia (C2, C3 and C4) and spinal cord were removed and assayed for infectious virus. The results from animals depleted before infection are shown in Fig. 5 and Fig. 6. In Fig. 5 the amount of virus recovered from the ear is shown. Mice deficient in Lyt 2 cells were well able to eliminate virus at a rate comparable to the untreated controls. However, depletion of L3T4 or both L3T4 and Lyt 2 cells resulted in a delayed clearance; in the latter case, the animals began to die by day 9. Not surprisingly, mice deficient in L3T4 or total T cells showed a more florid infection of the peripheral and central nervous system. However, removal of Lyt 2 cells also led to a more severe infection in the ganglia and spinal cord (Fig. 6) without any increased mortality. On the basis of these results it appears that Lyt 2 cells are more active in restricting virus infection in the nervous system, but do not appear to contribute significantly to the elimination of virus in the skin, at least with the dose of virus used in these experiments.

DISCUSSION

Monoclonal antibodies specific for the L3T4 and Lyt 2 antigens on T cells have been used here to analyse the immune response of mice to HSV. From the results of this study a number of
interesting features have emerged. First, in L3T4-deficient animals cytotoxic T cell responses are still induced and even augmented above that observed in untreated mice. Secondly, despite the presence of cytotoxic T cells, the clearance of infective virus from the ear and nervous system is delayed; such animals have an increased mortality. Thirdly, in Lyt 2-deficient mice, cytotoxic T cells are abolished but virus is cleared from the ear normally, though the elimination of virus from the peripheral and central nervous system is markedly delayed.

The phenomenon associated with the induction of cytotoxic T cell responses in 'helperless' mice is novel, if somewhat unexpected. The dogma on the induction of Lyt 2 cytotoxic T cell responses \textit{in vitro} clearly implicates L3T4 (Lyt 1) helper cells as providing the necessary signals for activating cytotoxic T lymphocyte precursors to their aggressor status (Cantor & Boyse, 1975; Wagner & Rollinghoff, 1978). Other groups have demonstrated departures from this dogma and have suggested that Lyt 2 cells provide their own source of 'helper' factors, or else receive induction signals from other cells (Andrus \textit{et al.}, 1981; Mizuochi \textit{et al.}, 1985; Moldwin \textit{et al.}, 1986; Sprent & Schaefer, 1986). Whatever the mechanism involved in Lyt 2 cell activation, it is clear that a deficiency in L3T4 cells actually leads to an augmented cytotoxic T cell response to this virus. Under these circumstances it is tempting to speculate that the Lyt 2 cell population is freed of regulation exerted by L3T4 T cells. In order to test this hypothesis we mixed cell cultures deficient in L3T4 (i.e. Lyt 2-positive) with cultures deficient in Lyt 2 (i.e. L3T4-positive) both obtained from infected mice. The result shown in Fig. 4 indicates that cytotoxic T cell responses were unaffected by the added L3T4 cells. It is possible that such cells could still have regulated the induction of precursor cytotoxic T cells \textit{in vivo}. Whatever the explanation for these observations it is clear from Fig. 5 that animals deficient in L3T4 function are more susceptible to herpesvirus infection, implying that Lyt 2 cytotoxic activity alone is not sufficient to control the early stages of an acute cutaneous herpesvirus infection.

In mice deficient in Lyt 2 cells, the virus-specific antibody response and delayed hypersensitivity response remain intact, whereas, cytotoxic T cell activity is abolished. In this environment the virus is cleared from the ear pinna by day 9, a result comparable to that seen in animals not treated with antibody. This suggests that L3T4 cells are active in eliminating virus from this site, a fact supported by previous observations involving the adoptive transfer of herpesvirus-specific Lyt 1\textsuperscript{+} 2\textsuperscript{−} lymph node cells or the local transfer of L3T4\textsuperscript{+}, Lyt 1\textsuperscript{+}, Lyt 2\textsuperscript{−} T cell clones (Leung \textit{et al.}, 1984; Nash & Gell, 1983). Furthermore, the \textit{in vivo} depletion experiments demonstrate that this protection occurs in the absence of any activity of cytotoxic T cells (Lyt 2 type). This is an important observation since with straightforward adoptive transfer experiments it could be concluded that L3T4 cells protect mice by helping in the induction of class I-restricted killer T cells.

Although virus clearance in the ear of Lyt 2-deficient animals was normal, the clearance of virus from the nervous system was markedly delayed. Analysis of both the sensory ganglia and the spinal cord revealed elevated virus titres in excess of those for both untreated and L3T4-deficient mice. An interesting feature of Lyt 2-deficient animals infected with 10\textsuperscript{4} p.f.u. SC16 is that there is no increase in mortality despite the elevated virus titres in the nervous system. This suggests that these mice eventually control the infection as indicated on day 9 in the dorsal root ganglia (see Fig. 6b). However, if the dose of virus injected is increased to 10\textsuperscript{5} and 10\textsuperscript{6} p.f.u. SC16 then an increased mortality is observed compared with that of normal controls, presumably due to an uncontrolled infection of the nervous system (A. A. Nash & A. Jayasuriya, unpublished observations).

It would appear from these experiments that immune T cells exert differential anti-herpesvirus activity in different target tissues, at least in the acute infection. In the skin, L3T4 cells act as the principal antiviral T cell, whereas in the nervous system, Lyt 2 cells assume a more significant role. The fact that both major T cell populations are active in the elimination of acute HSV infections is supported by results obtained from MHC matching experiments between donor immune T cells and recipient infected mice (Howes \textit{et al.}, 1979; Nash \textit{et al.}, 1981). In the report by Nash \textit{et al.} (1981) the rapid elimination of HSV was achieved only when recipient and donor were compatible both at the H-2\textsuperscript{k} (D) (class I) and at the H-2 IA (class II) regions.
The observations reported here indicate the importance of T cell depletion in vivo ("immunosurgery") as a technique for studying the immunology of infectious diseases. Although used here to study the pathogenesis and immunology of an acute virus infection, the technique should be particularly relevant to studying chronic virus infections, where immune mechanisms are implicated in the persistence of viruses. Furthermore, the contribution of T lymphocytes to the pathology of virus diseases, particularly in the central nervous system, is still poorly understood. The application of these monoclonal antibodies would greatly increase our understanding of the role played by T cells in virus-induced immune pathology.

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