Murine CD4⁺ T cell clones vary in function in vitro and in influenza infection in vivo

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Abstract

Several CD4⁺ Tₕ₁ clones specific for Influenza haemagglutinin or nucleoprotein were transferred into syngeneic mice after intranasal influenza infection to examine whether they accelerate viral clearance in vivo similarly to CD8⁺ cytotoxic T cells. We observed changes in functional properties of the CD4⁺ clones in vitro and variable effects on the course of infection in vivo. While some clones resulted in more rapid virus clearance, others had no protective effect, but rather exacerbated illness symptoms. Our results reflect problems in the in vivo use of CD4⁺ T cell clones maintained in long-term culture. Their IL-2 and IL-5 release and cytolytic activity varied, while IL-3 and γ-IFN production as well as DTH induction were more stable. CD4⁺ T cells primed by infection became cytolytic only after prolonged culture. The data point to the fine balance between exacerbation of disease and protection by CD4⁺ T cells.

Introduction

Cloned influenza specific cytotoxic T cells (CTL) which are CD8⁺ and class I MHC restricted can protect against lethal influenza infection of mice and accelerate clearance of lung virus by day 6 (1 – 3). This effect is reflected by a more rapid recovery from lung pathology (4). Since CD4⁺ T cell responses are far more easily induced than CD8⁺ CTL by subunit vaccines, we wished to know whether CD4⁺ T cells played a role in heterotypic immunity following influenza infection by accelerating viral clearance similarly to CD8⁺ CTL (1 – 3). Antigen stimulation induces the secretion of similar levels of γ-IFN by both T cell subpopulations (Tₕ₁ cells and CD8⁺ T cells) (e.g. 5), but the two T cell subsets differ in other functions, such as the levels of interleukin release, DTH induction, and cytolytic activity.

Previous experiments with CD4⁺ cells have been very limited. A single HA-specific clone transferred into nu/nu mice amplified antibody responses and resulted in viral clearance by 2 weeks of infection (6). A neuraminidase-specific clone (7) or primed spleen cells selected for expression of Lyt-1 led to survival of mice given a lethal influenza challenge (8). However, the short-term effector function of CD4⁺ cells had not been examined. In earlier experiments we found that in vivo depletion of CD8⁺ T cells by monoclonal antibodies (9) was not sufficient to abolish the generation of significant CD8⁺ CTL responses to influenza (10). Therefore in the present study we transferred several CD4⁺ and influenza-specific T cell clones into infected hosts to observe their short-term effect on virus replication in the lung of influenza infected syngeneic mice. Our results emphasize the difficulties arising in the use of CD4⁺ T cell clones in long-term culture to study in vivo function. The effector function in vivo varied between individual Tₕ₁ clones and within the life of a single clone with time in culture and changes in interleukin secretion or cytolytic activity could be observed.

Methods

Mice

BALB/c a mice, aged 3 – 5 months, were bred under specific pathogen-free conditions at NIMR, Mill Hill, London.

Influenza virus and proteins

Influenza viruses A/X31 (H3N2 recombinant with H1N1 internal proteins) and A/PR/8/34 (H1N1) were grown in the allantoic cavity of 10-day-old embryonated chicken eggs, and stored at -70°C as infectious allantoic fluid. Virus was inactivated by irradiation at a distance of 10 cm from a 15 W UV lamp for 10 min. Purified HA from bromelain digested A/X31 (11) was kindly provided by D. Stevens (NIMR). The remaining viral cores were treated with ammonium deoxycholate (12) to precipitate the matrix protein and to allow purification of nucleoprotein (NP) (13).

Selection and maintenance of CD4⁺ T cell clones

The general method has been described by Mills et al. (14). In brief, BALB/c mice were infected i.n. with A/X31 virus (2.5 HAU). After 2 – 4 months spleen cells were cultured at 1 – 2 × 10⁴/ml,
with UV inactivated A/AX31 unless otherwise indicated. Four days later cells were cloned by limiting dilution in the presence of virus and APC (normal spleen cells, 2000 rad, at 5 x 10^6/well) with a source of IL-2 (supernatant from 48 h stimulation of rat spleen cells with Con A at 5 μg/ml). Clones were maintained in 25 cm² flasks by the following regime: every 10–12 days 2 x 10^5 cells/ml were stimulated with antigen and APC at 2 x 10^6 cells/ml. After 3–5 days, 5% IL-2 was added to the cultures.

This method of selection proved ineffective in obtaining NP-specific CD4⁺ clones. Clone 2F3 was selected by using purified NP instead of A/AX31 for the cloning procedure. For clone 13.3, donor spleen cells were depleted in vitro with C and anti-Lyt-2 mAb HO2.2 (15) and J11d (anti-lg; 16), and cells were cloned directly using UV-inactivated A/PR/8 virus as the antigen. Both NP-specific clones grew optimally with NP or less pure virus core (1 μg/ml). The time of our culture of clones was calculated starting at 2 weeks after cloning, i.e. once reliable clonal growth had occurred, it does not necessarily reflect continuous growth, as clones at times were frozen and thawed.

Antigen recognition assays
Clones were tested for antigen specificity 10–12 days after antigen stimulation (14). Tₙ cells (10⁴) were cultured with 4 x 10⁵ APC and purified antigen (0.1–10 μg/ml) in 200 μl medium in 96-well microplates. After 48–72 h cells were pulsed with 5 μCi [³H]thymidine ([³H]TdR) for 4–6 h before harvesting.

T cell transfer and lung virus titration
Mice were infected i.n. with 4–5 HAU A/AX31 virus (3–4 mice group). After 1–2 h, CD4⁺ T cells (in BSS with 5% haemaccel, Hoechst, Hounslow, Middlesex) were mixed with recombinant human IL-2, generously provided by Hoffman La Roche (Basel), and transferred i.v. into the infected hosts (5000 units/mouse). On days 6–7 post-infection, lungs within a group were pooled and homogenized before dilution and virus titration in the allantoic cavities of 10-day-old chick embryos. Infectivity titres are expressed as EID₅₀ in log₁₀ terms (1).

Class II MHC restricted target cell lysis
Target cells were the A20.2J B cell lymphoma line (17) (kindly donated by J. Tite, Wellcome Labs, Beckenham, Kent), which expresses both class I and class II MHC molecules on its surface. Following a 1 h incubation with infectious A/AX31 virus (1000 HAU/10⁵ cells) and ⁵¹Cr (100 μCi/10⁵ cells), cells were washed and a 6 h ⁵¹Cr release assay performed as described previously (18).

Interleukin assays
The cloned CD4⁺ cells (2–5 x 10⁶/ml) were washed and, 10–12 days after antigen stimulation, stimulated with UV-inactivated A/AX31 virus at 100 HAU/ml, HA, NP, or influenza core protein (1 μg/ml), and syngeneic, normal spleen cells (irradiated at 2000 rad) as APC. Following incubation at 37°C, cell-free supernatants were stored at −20°C and interleukins assayed (see below). The stimulation index (SI) was calculated as follows:

\[ \text{SI} = \frac{\text{cpm cell supernatants; no antigen}}{\text{cpm cell supernatants; antigen stimulated}} \]

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Interleukin assays relating to a single clone at various times of culture were carried out on the same day.

IL-2
Two-fold dilutions of cell supernatants (50 μl/well) were tested in triplicate in RPMI/5 in round-bottom 96-well microtitre plates. CTLL cells, maintained in RPMI/10 with a source of IL-2, were washed three times before plating out at 10⁴ cells in 50 μl in RPMI/5. Each assay included control supernatants (no antigen) and a known IL-2 supernatant. Following a 20 h incubation period at 37°C, cells were pulsed with [³H]TdR (0.5 μCi/well) for 4 h.

Cells were then harvested and processed for scintillation counting.

IL-3
32D cells (19) were maintained in RPMI/10 and 5% WEHI3 supernatant [obtained from dense cultures of WEHI3 cells (20)]. The assay conditions for IL-3 are essentially identical to the method described above for IL-2, but using 32D cells for the read out.

IL-5
Frozen stocks of T cell-depleted BCL₃ lymphoma cells (21) were generously provided by Dr Arturo Gonzalez (NIMR). Two-fold dilutions of supernatants were tested in triplicate in RPMI/5, in flat-bottom microtitre plates. Thawed, washed BCL₃ cells were plated out at 2.5 x 10⁴ cells/well (total volume 200 μl/well). Plates were incubated at 37°C for 44 h, and cells then pulsed with [³H]TdR (0.5 μCi/well) for 4 h before harvest and scintillation counting. BCL₃ cells can also respond to IL-2, thus IL-5 could only be assessed in the absence of IL-2.

γ-IFN
The γ-IFN content in supernatants from antigen-stimulated Tₙ cells was kindly assayed by Dr A. Meager (NIBSC, South Mimms) using a highly specific radioimmunooassay which utilizes two mAbs specific for γ-IFN (5). All results were standardized with the murine γ-IFN standard NIH Gg02-901-533.

Delayed type hypersensitivity assay
Tₙ clones in varying cell numbers were injected with 5 μg of purified A/AX31 virus in a volume of 30 μl into the right hind footpads of four mice per group. An equal volume of PBS was injected into the left hind footpad. Footpad thickness was measured at 24 h with dial calipers (Pocotest, FRG).

% footpad increase = \[ \frac{\text{right (experimental) - left (PBS)}}{\text{normal footpad}} \times 100 \]

The low increases in footpad size seen at control sites (virus or cells alone) were subtracted.

Results
Characterization of CD4⁺ T cell clones
The phenotype of the clones used in this study was ascertained by fluorescent antibody staining [anti-L3T4, mAb YTS 191.1; anti-Lyt-2, mAb YTS 169.4 (9)]. No Lyt-2⁺ cells were detected and 90% of the cells were L3T4⁺. This phenotype was stable.
Influenza specific CD4+ T cell clones

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during the entire culture time. We refer to these cells as CD4+ T cells or Tp. Antigen specificity was established by stimulating the cloned cells with different concentrations (0.1 – 10 μg/ml) of purified H3 (from X31 virus) or NP (derived from A/PR/8/34 virus) as described by Mills et al. (14). On antigen stimulation, all the clones when first selected secreted IL-2 and IFN (not illustrated) and belonged to the Tp1 subset (22). Anti-IL-2 (mAb S4B5, 1/500 ascites) was shown to totally block CTLL proliferation to confirm that the lymphokine released was IL-2 (Fig. 1). Two BALB/c NP-specific and A virus cross-reactive clones 2F3 and 13.3 and H3-specific clone 2A12 were derived by F.E., and three other BALB/c H3-specific CD4+ clones (BAE5, BA 5.2, BA 5.6) were kindly given by D. B. Thomas and C. Graham, NIMR (23).

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Functional variation of HA-specific CD4+ T cell clones

In pilot experiments we transferred a CD4+ influenza-specific T cell line into sublethally irradiated mice infected with X31 virus. Three to four million cells were lethal to the hosts within a few days, while the infected mice without T cells survived. This prompted us to transfer newly available HA-specific clones into infected but not irradiated syngeneic mice. To obtain sufficient numbers of cells long-term culture was necessary. Table 1 illustrates that individual clones specific for H3, the haemagglutinin of the X31 virus, differed in their effects in vivo. Transfer of two of the clones (6–9 x 10^6 cells) exacerbated illness symptoms and had no significant effect on lung virus titres. One of them (clone 5.6) appeared to slightly enhance lung virus titres. In contrast, transfer of the two other H3-specific clones at similar cell numbers led to a reduction in virus recoverable from the lung on day 6. At the time of cell transfer, these clones were able to lyse A20.2J targets infected with X31 virus but at much lower efficiency than our CD8+ CTL Clones 2A12 and BAE 5 were also tested for DTH induction (20–30% increase in footpad thickness by 2.5 x 10^5 cloned cells). The other two clones had not been tested for cytolytic ability at the time of the cell transfers.

These experiments showed that CD4+ T cell clones recognizing the same viral protein vary in their effector function in vivo and we wondered whether this might be attributable to changes in interleukin production or other properties between clones during their long-term maintenance in vitro. The next clones selected were analysed accordingly at the time of the cell transfers.

Transfer of CD4+ NP-specific T cell clones

We were particularly interested to examine whether influenza A virus cross-reactive CD4+ T cells were able to accelerate viral

Fig. 1. Antigen-induced IL-2 production by CD4+ T cell clone 2A12 6 weeks after cloning. Proliferation of CTLL in the presence of supernatant of A/X31 stimulated 2A12 cells (•—•); this is totally inhibited by 1/500 dilution of mAb S4B6 (anti-IL-2) ascites (O—O).

Table 1. HA-specific CD4+ T cell clones differ in their in vivo effect

| Clone | Weeks in culture | Cell transfer (no. x 10^6) | Lung virus titre (dil), log_{10} EID_{50} | % A/X31-specific A20-2J lyss a | % A/X31-specific footpad b |
|-------|------------------|-----------------------------|------------------------------------------|------------------------------|----------------------------|
| BA5 2 | 18               | –                           | 5.2                                      | ND                          | ND                         |
| BA5 6 | 24               | 9                           | 4.8 c                                    | ND                          | ND                         |
| BA5 6 | 24               | 6                           | 5.2                                      | ND                          | ND                         |
| 2A12  | 9                | 6                           | 6.2 c                                    | 15                          | 33                         |
| 2A12  | 14               | –                           | 4.5                                      | 24                          | 33                         |
| 2A12  | 15               | –                           | 5.5                                      | 31                          | 26                         |

The H3-specific CD4+ T cell clones were BALB/c derived. Cells were transferred i.v into syngeneic mice 2 h after i.n. A/X31 infection and lung virus titred on day 6 of infection.
aK/T = 10. b2.5 x 10^5 cloned cells/footpad. cIncreased sickness symptoms.
Influenza specific CD4+ T cell clones

Table 2. NP-specific CD4+ T cell clones can enhance disease or recovery

| Clone | Weeks in culture | Cell transfer (no. × 10^6) | Lung virus titre (d6), log_{10} EID_{50} | % A/X31-specific A20-2J lysis | DTH% | % A/X31-specific footpad increase |
|-------|------------------|----------------------------|---------------------------------|--------------------------------|------|----------------------------------|
| 13 3  | 8                | –                          | 4.5                             | ND                             | ND   | ND                               |
|       | 10               | –                          | 4.5                             | ND                             | ND   | ND                               |
|       | 13               | –                          | 4.5                             | 27                             | 11   |                                  |
| 2F3   | 10               | –                          | 4.5 (0/3 died)                  | ND                             | ND   | ND                               |
|       | 18               | –                          | 5.5                             | 0                              | 20   |                                  |
|       | 21               | –                          | 4.5                             | 22                             | ND   |                                  |

BALB/c CD4+ T cell clones were transferred i.v. 2 h after A/X31 i.n. infection of hosts

* K/T = 10.
* 2.5 × 10^5 cloned cells
* Mice very ill.

Clearance similarly to CD8+ T cell clones and possibly to play a part in partial heterotypic protection. We were able to select two NP-specific and A virus cross-reactive CD4+ clones which enabled us to study several parameters over several weeks of culture: antigen-induced interleukin production in vitro, cytolytic capacity, and in vivo function (DTH induction and viral clearance).

Clone 13.3 grew well and was first tested after 8 weeks of culture. Transfer of only 5 million cells enhanced sickness symptoms in comparison to control-infected mice; within several days of infection and cell transfer the host mice were severely ill (sweating, ruffled fur, huddling, and tachypnoic signs), though the lung virus titers were the same as in the control-infected mice. After another 2 weeks of culture even higher numbers of cells (8 × 10^6) did not affect viral replication in the lung, while after 13 weeks of culture the same number of cloned cells transferred resulted in a two-log reduction of lung virus titre by day 6. Interleukin production had changed during the interval between transfers. IL-2 release was not detectable any more by 13 weeks of culture, while IL-3 production had increased. At the time of protective activity clone 13.3 was able to lyse X31-infected A20 2J target cells (Table 2 and Fig. 2).

The second NP-specific clone 2F3 was transferred first after 10 weeks in culture; again 5 × 10^6 cells made the host mice very ill and the lung virus titre was slightly higher than in the control mice 6 days post-infection. One-third of the mice died by day 5. After a few more weeks of culture, a similar effect could be observed. Another test after 21 weeks of culture did not exacerbate illness symptoms and no effect was noted on the lung virus titre when 8 × 10^6 cloned cells were transferred (Table 2). With this number of 13.3 cells a two-log reduction in lung virus resulted.

Lymphokine assays at the time of cell transfer are illustrated in Fig. 2. IL-2 production was down-regulated by 18 weeks in culture, at which time IL-5 induction was detectable. The only difference between the last two transfers of clone 2F3 was an increase in cytolytic activity. This clone could not lyse the influenza-infected targets at the time of the first two cell transfers.

Fig. 2. Antigen-induced IL release by CD4+ clones. Supernatants collected 24 h after antigen stimulation of NP-specific clones 13.3 and 2F3 were tested for interleukin activity by proliferation of CTLL (IL-2), 32D (IL-3), or BCL1 cells (IL-5).

\[
S_I = \frac{cpm \text{ in antigen stimulated SN}}{cpm \text{ SN (no antigen)}}
\]

DTH responses induced by the protective and non-protective clones were of similar magnitude, and both clones continued to produce high levels of γ-IFN (1000–2600 units/ml/5 × 10^5 cells) in the presence of antigen (not illustrated).
Our data show very clearly that CD4+ T cells can vary in their in vitro and in vivo functions during long-term culture but at present it is not yet possible to correlate individual functional changes with the observed effects.

Discussion

Influenza-specific CD4+ T cell clones, described above, early after selection produced IL-2, IL-3, and γ-IFN in response to antigen and thus belonged to the T_h subset (22). This subset is also considered to be inflammatory and to induce strong DTH reactions (24,25).

Our present results emphasize the difficulties that arise in the use of CD4+ T cell clones for functional studies in vivo since their properties in vitro and in vivo can change with time in culture. The variable effects of several influenza-specific CD4+ T cell clones on lung virus replication and exacerbation of illness symptoms that we observed does not correlate with viral specificity (HA or NP) of the T cells. Not only do different clones vary in their effects, but a given clone can change with time in culture in regard to antigen-induced interleukin production, cytolytic activity and in vivo effector function. Our clones continued to secrete γ-IFN (not illustrated) and IL-3 on antigen contact during many weeks of culture, but IL-2 production by all clones was down-regulated in vitro while IL-5 production became evident with time IL-5 production by T_h cells has been previously reported (25). CD4+ memory T cells primed by i.n. influenza infection of mice are not cytolytic when stimulated with antigen for several days (F. Esquivel, unpublished results); however, our CD4+ T cell clones acquired cytotoxic capacity to a variable degree in tissue culture, as also reported for human T cells by Fleischer (26). In contrast, Tate et al. (27) reported that rNP in adjuvant induces CD4+ memory cells that become cytolytic within a few days of culture.

Since HA-specific T_h cells are virus subtype specific, our main interest concerned the A virus cross-reactive NP-specific CD4+ T cells. Clone 13.3 started to accelerate viral clearance after > 10 weeks in vitro when cytotoxicity was found but IL-2 production was not detectable. Clone 2F3 never protected—up to 18 weeks of culture it exacerbated illness symptoms and, if anything, enhanced virus replication rather than reducing it: At that stage it was not cytotoxic and IL-2 production had stopped. Later, 2F3 cells were able to lyse infected target cells and did not affect lung virus titres. Preliminary analyses of lung pathology in influenza-infected mice after transfer of CD4+ clones (with Dr C. D. Mackenzie) suggest that in contrast to the rapid recovery of the epithelium seen with our CD8+ CTL clones (4), early recovery of epithelium is not evident after transfer of CD4+ clone 2F3. This clone induced a mixed cellular infiltrate, including macrophages and polymorphonuclear cells, in contrast to CD8+ clones, which induce a predominantly lymphocytic infiltrate (4).

Detailed analysis of the lung histology will be required to understand how some CD4+ T cell clones might exacerbate illness, strong inflammatory responses may well lead to enhanced lung pathology. CD4+ T cells secrete numerous mediators, new ones are still being discovered, and with further knowledge one hopes to understand which CD4+ T cell-derived mediators would be responsible for the variation in effector function of CD4+ T cell clones after prolonged culture. Differences in the role of T_h1 and T_h2 cells have been observed, for example, in Leishmaniasis (28,29). As the role of T cell subpopulations is being analysed in more infections, it becomes clear that a given T cell subpopulation (be it CD8+ or CD4+) can exert either beneficial effects or exacerbate immunopathology and illness in different virus infections or the same infection depending on the site of infection, i.e. LCMV (30,31). Quantitative differences were observed in respiratory syncytial virus infection of mice. Low numbers of transferred CD8+ T cells were helpful, while high numbers augmented pathology (32).

Our present observations indicate that CD4+ T cells are less effective in early influenza virus clearance than CD8+ T cells. Coronavirus-specific CD4+ T cell clones also were poor in viral clearance (33). In spite of migration problems, results with our CD8+ CTL clones have been far more consistent than with the CD4+ clones, presumably due to the many more mediators produced by CD4+ cells. Continuous detailed functional analyses will be required at the time of clonal transfers to define protective or deleterious effects in vivo by CD4+ T cells. Our results point to the fine balance that can exist between exacerbation of illness or enhanced viral clearance by virus-specific T cells.

Abbreviations

CTL cytotoxic T cells
T_h T-helper cells
HA, NP haemagglutinin and nucleoprotein of influenza virus
HAU haemagglutination units
DTH delayed type hypersensitivity
γ-IFN γ-interferon
RP/M10 RPMI 1640 + 10% fetal calf serum
TdR thymidine

References

1 Lin, Y. L. and Askonas, B. A. 1981. Biological properties of an influenza virus specific T cell clone. J. Exp. Med. 54:225
2 Lukacher, A. E., Braciale, V. L., and Braciale, T. J. 1984 In vivo effector function of influenza virus specific cytotoxic T lymphocyte clones is highly specific. J. Exp. Med. 158:814.
3 Taylor, P. M and Askonas, B. A. 1989. Influenza virus specific helper T cells are protective in vivo. Immunology 59:417.
4 MacKenzie, C. D., Taylor, P. M., and Askonas, B. A. 1989. Rapid recovery of lung histology correlates with clearance of influenza virus by specific CD8+ cytotoxic T cells. Immunology 67:375.
5 Taylor, P. M., Meager, A., and Askonas, B. A. 1989. Influenza virus specific T cells lead to early interferon in lungs of infected hosts: development of a sensitive radioimmunoassay. J. Gen. Virol. 70:975.
6 Garbard, W., Hackett, C., and Melchers, F. 1983. The recognition specificity of a murine helper T cell for haemagglutinin of influenza virus A/PR/8/34. J. Immunol. 130:2379.
7 McDermott, M. R., Lukacher, A. E., Braciale, V. L., Braciale, T. J., and Bienstock, J. 1987. Characterization and in vivo distribution of influenza virus specific T lymphocytes in the murine respiratory tract. Am. Rev. Respir. Dis. 135:245.
8 Leung, K. N. and Ada, G. L. 1982. Different functions of subsets of effector cells in murine influenza virus infection. Cell Immunol. 67:312.
9 Cobbold, S., Jajasanta, A., Nash, A., Prospero, T. D., and Waldmann, H. 1984. Therapy with monoclonal antibodies by elimination of T cell subsets in vivo. Nature 312:548.
10 Lightman, S., Cobbold, S., Waldmann, H., and Askonas, B. A. 1987. Do L3T4+ T cells act as effector cells in protection against influenza virus infection? Immunology 62:139.
11 Brand, C. M and Skehel, J. J. 1972. Crystalline antigen from the
Influenza specific CD4+ T cell clones

12 Laver, W.G. and Webster, R.G. 1976. Preparation and immunogenicity of an influenza virus haemagglutinin and neuraminidase subunit vaccine. Virology 69: 511.

13 Wraith, D.C. and Askonas, B.A. 1985. Induction of influenza A virus cross-reactive cytotoxic T cells by a nucleoprotein/haemagglutinin preparation. J. Gen. Virol. 66:1327.

14 Mills, K.H.G., Skehel, J.J., and Thomas, D.B. 1986. Extensive diversity in the recognition of influenza virus haemagglutinin by murine T helper clones. J. Exp. Med. 163:1477.

15 Gottlieb, P.D., Marshak, R.A., Auditore, H.K., Borkoben, D.B., August, D.A., Rosche, R.M., and Benedetto, J.D. 1980. Construction and properties of new Lyt congenic strains and anti-Lyt2.2 and anti-Lyt3.1 monoclonal antibodies. Immunogenetics 10:545.

16 Bruce, J., Symington, F.W., McKearin, D.J., and Sprent, J. 1981. A monoclonal antibody discriminating between subsets of T and B cells. J. Immunol. 127:2496.

17 McKeon, D.J., Infante, M.J., Nelson, A., Fathman, C.G., Walker, G., and Warner, N.L. 1981. Major histocompatibility complex restricted antigen presentation to antigen reactive T cells by B lymphocyte tumour cells. J. Exp. Med. 154:1419.

18 Zweerink, H.J., Askonas, B.A., Millican, D., Courrède, S.A., and Skehel, J.J. 1988. Cytotoxic T cells to type A influenza virus haemagglutinin induce A strain specificity while infected cells confer crossreactive cytotoxicity. Eur. J. Immunol. 18:763.

19 Greenberger, J.S., Sakakeeny, M.A., Humphries, R.K., Eaves, C.J., and Eckner, R.J. 1983. Demonstration of permanent factor dependent multipotential (erythroid/neutrophil/basophil) hematopoietic progenitor cell lines. Proc. Natl. Acad. Sci. USA 80:2931.

20 Lee, J.C., Hapel, A.J., and Ihle, J.N. 1982. Constitutive production of a unique lymphokine (IL-3) by the WEHI-3 cell line. J. Immunol. 128:2393.

21 Savin, S. and Strober, S. 1978. Spontaneous murine B cell leukaemia. Nature 272:624.

22 Mosmann, T.R., Cherwinski, H., Bond, M.W., Giedlin, M.A., and Coffman, R.L. 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphocyte activities and secreted proteins. J. Immunol. 136:2348.

23 Thomas, D.B., Skehel, J.J., Mills, K.H.G., and Graham, C.M. 1986. Suicide selection of murine T helper clones specific for variable regions of influenza haemagglutinin molecule. Eur. J. Immunol. 16:789.

24 Cher, D.J. and Mosmann, T.R. 1987. Two types of murine helper T cell clone. II. Delayed type hypersensitivity is mediated by Th1 clones. J. Immunol. 138:3669.

25 Janeway, C.A., Carding, S., Jones, B., Murray, J., Pilar, P., Rasmussen, R., Rop, J., Sazawa, K., West, J., and Bottomly, K. 1988. CD4+ T cells specificity and function. Immunol Rev. 101:55.

26 Fleischer, B. 1984. Acquisition of specific cytotoxic activity by human T4+ lymphocytes in culture. Nature 308:365.

27 Tate, J.P., Russell, S.M., Dougan, G., O’Callaghan, D., Jones, I., Brownlee, G., and Liew, F.Y. 1988. Anti-viral immunity induced by recombinant nucleoprotein of influenza A virus. I Characteristics and cross reactivity of T cell responses. J. Immunol. 141:3980.

28 Scott, P., Natowitz, P., Coffman, R.L., Pearce, E., and Sher, A. 1988. Immunoregulation of cutaneous leishmaniasis. T cell lines that transfer protective immunity or exacerbation belong to different T helper subsets and respond to distinct parasite antigens. J. Exp. Med. 168:1675.

29 Liew, F.Y. 1989. Functional heterogeneity of CD4+ T cells in leishmaniasis. Immunol. Today 10:40.

30 Byrne, J.A. and Oldstone, M.B.A. 1986. Biology of cloned cytotoxic T lymphocytes specific for lymphocytic choriomeningitis virus. J. Immunol. 136:698.

31 Baenziger, J., Hengartner, H., Zinkernagel, R.M., and Cole, G.A. 1986. Induction or prevention of immunopathological disease by cloned cytotoxic T cell lines specific for lymphocytic choriomeningitis virus. Eur. J. Immunol. 16:387.

32 Cannon, M.J., Openshaw, P.J.M., and Askonas, B.A. 1988. Cytotoxic T cells clear virus but augment lung pathology in mice infected with respiratory syncytial virus. J. Exp. Med. 168:1163.

33 Stohlman, S.A., Matsushima, G.K., Casteel, N., and Weiner, L.P. 1986. In vivo effects of coronavirus specific T cell clones. DTH inducer cells prevent a lethal infection but do not inhibit virus replication. J. Immunol. 136:3052.