Clin. exp. Immunol. (1983) **53**, 25–30.

Monoclonal antibody defined T cell subsets and autologous mixed lymphocyte reaction in Down's syndrome

S. GUPTA, S. M. FIKRIG,* E. MARIANO* & Q. QUAZI* *Division of Basic and Clinical Immunology, Department of Medicine, University of California, Irvine, California and* **Department of Pediatrics, Downstate Medical Center, Brooklyn, New York, USA*

(Accepted for publication 4 February 1983

SUMMARY

Peripheral blood from 21 non-institutionalized children with Down's syndrome (DS) and 21 age and sex matched simultaneously studied healthy controls, was analysed for monoclonal antibody defined T cells and T cell subsets, using a fluorescence activated cell sorter, and the autologous (AMLR) and allogeneic mixed lymphocyte reaction (MLR). Total T cells ($9{\cdot}6^+$), $OKT4^+$ (helper/inducer phenotype) and anti-Tac^+ (activated T) cells were present in comparable proportions to that observed in the control group. In contrast, the proportion of $OKT8^+$ (suppressor/cytotoxic phenotype) cells were significantly ($P < 0{\cdot}05$) decreased when compared to healthy controls. The proliferative response in the AMLR and MLR were comparable to control group. The significance of these results are discussed.

Keywords monoclonal antibodies T cell subsets Down's syndrome MLR

INTRODUCTION

Down's syndrome (DS) is considered, at least partially, a model for accelerated ageing in man (Martin, 1977). Immunological abnormalities include: decreased antibody production *in vivo* (Burgio & Ugazio, 1978; Lopez *et al.*, 1975), normal or decreased number of B lymphocytes (Frenchesi *et al.*, 1978; Levin *et al.*, 1979) decreased numbers of T cells (Frenchesi *et al.*, 1978; Levin *et al.*, 1979; Gershwin *et al.*, 1977; Ugazio *et al.*, 1977), decreased delayed cutaneous hypersensitivity to recall antigens (Sutnik *et al.*, 1971), depressed proliferative response to mitogens (Frenchesi *et al.*, 1978; Sutnik *et al.*, 1971; Rigas, Elasser & Hecht, 1970; Burgio *et al.*, 1975; Walford *et al.*, 1981) and alloantigens (Walford *et al.*, 1981), involution of thymus (Levin *et al.*, 1979) and decreased levels of thymic factors (Frenchesi *et al.*, 1981), and increased incidence of autoantibodies (Burgio & Ugazio, 1978; Gershwin *et al.*, 1977; Whittingham *et al.*, 1977; Fialkow, 1970). There have been however, some inconsistencies in the results of immunological studies among investigators. This appears primarily due to the lack of age and sex matched simultaneously studied healthy controls in some studies. Further, most of the immunological data are reported on institutionalized patients that could be different from non-institutionalized patients with DS. Recently, human T cells and T cell subsets have been defined with murine monoclonal antibodies (Reinherz & Schlossman, 1970). $OKT4^+$ T cells contain cells with helper/inducer phenotype and $OKT8^+$ monoclonal antibody reacts with cytotoxic/suppressor phenotype cells. In the autologous mixed lymphocyte reaction (AMLR), $OKT4^+$ T cells proliferate upon stimulation with autologous non-T cells (Damle *et al.*, 1981). Deficiency of $OKT8^+$ T cells and of AMLR has been considered a possible mechanism for the autoimmune manifestation of human ageing (Moody *et al.*, 1982). We, therefore, analysed circulating T cells and T cell subsets, using monoclonal antibodies and fluorescence activated cell

Correspondence: Professor Sudhir Gupta, Division of Basic and Clinical Immunology, Department of Medicine, University of California, Irvine, California 92717, USA.

sorter (FACS), and the proliferative response of T cells in the AMLR in 21 non-institutionalized children with DS.

MATERIALS AND METHODS

Twenty-one non-institutionalized children with DS (age range 2–12 years, mean age 5 years) were the subject of this study. Twenty-one age and sex matched controls were simulataneously studied. All patients were karyotyped and had trisomy 21.

Mononuclear cells were isolated on Ficoll-Hypaque (F H) density gradient. Cells were washed thrice with Hank's balanced salt solution (HBSS) and resuspended in HBSS at a desirable concentration. Mononuclear cells were used to separate T and non-T cells. Equal volumes of mononuclear cell suspension (3×10^6/ml of HBSS) were mixed with aminoethylisothiouronium treated sheep red blood cells (SRBC), incubated at the room temperature for 15 min, layered on F H gradient and centrifuged at 400*g* for 20 min at 22°C in a temperature controlled centrifuged. Rosetted T cells were separated from non-rosetted non-T cells. SRBC were lysed with tris buffer containing 0·83% ammonium chloride (pH 7·2) and washed three times with HBSS. Both T and non-T cells were resuspended in RPMI 1640 medium supplimented with 10% AB serum (Irvine Scientific, Santa Ana, California, USA), 100 u penicillin/ml, 100 μg streptomycin/ml, 10 μg gentamycin/ml and 2 mM L-glutamine (culture medium) at a concentration of 1×10^6 cells/ml. T cell fractions contained greater than 95% T cells as defined with monoclonal pan-T cell antibody 9·6 (Kamoun *et al.*, 1981). Non-T cell fractions contained less than 5% T cells, 60% surface Ig^+ B cells, 15–20% adherent, phagocytic, esterase positive and M2 monoclonal antibody (Bethesda Research Laboratory, Bethesda, Maryland, USA) reactive monocytes and 15–20% non-T, non-B (null cells) lymphoid cells. Non-T cells were irradiated at 3,000 rad from a Caesium source and used as stimulators in AMLR and allogeneic MLR.

Analysis of T cells and T cell subsets. OKT4 and OKT8 monoclonal antibodies were purchased from Ortho Diagnostics Raritan, New Jersey, USA; pan-T cell antibody, (9·6), was a gift from Dr John A. Hansen of Fred Hutchinson Cancer Center, Seattle, Washington, USA. Anti-Tac antibody that defines an antigen present on activated T cells was a gift from Dr T.A. Waldmann of National Cancer Institute, Bethesda, Development, specificity and characterization of these antibodies have been described elsewhere (Kamoun *et al.*, 1981; Uchiama, Broder & Waldmann, 1981). Mononuclear cells ($0{\cdot}5–1 \times 10^6$) were used for each antibody. Cells were washed with phosphate-buffered saline (PBS) and the cell pallets were mixed with 50 μl of appropriate dilutions of each 9·6, OKT4 and OKT8, and anti-Tac monoclonal antibodies. Cells were incubated at 4°C for 30 min, followed by three washings with PBS. Cells were then mixed with 25 μl of fluorescein isothiocyanate conjugated goat $F(ab')_2$ IgG anti-mouse IgG antibody (Cappel Laboratories, Cochraneville, Pennsylvania, USA) and incubated at 4°C for another 30 min. Cells were washed three times with PBS and resuspended in 0·5 ml of PBS. Quantitation of lymphocyte subpopulations was done by fluorescence activated cell sorter (FACS IV, Becton-Dickinson, Montainside, California). The absolute lymphocyte count in DS (mean ± s.d.; 2,149 ± 450) was comparable to controls (mean + s.d.; 2,642 ± 700), therefore, data of subset are expressed as percentage.

Autologous and allogeneic mixed lymphocyte reactions. One hundred microlitres of responder T cells (10^5/well) were cultured with 100 μl of equal number of irradiated autologous or allogeneic stimulator non-T cells in U bottomed microtitre plates (NUNC, Denmark). Responder T cells and stimulator non-T cells were cultured alone for spontaneous background proliferation. All cultures were performed in triplicate and incubated at 37°C in a humidified incubator with 5% CO_2 for 5, 6 and 7 days. The proliferative response was measured by pulsing cells with 1 μCi of ^{3}H-thymidine (New England Nuclear, Boston, Massachusetts, USA) for the final 16–18 h of cultures. The samples were harvested on glass fiber filters by an automated multiple sample harvester (Flow Laboratories, McLean, Viginia, USA), resuspended in a standard scintillation mixture, and counted in a liquid scintillation spectrometer. The results are expressed for the peak proliferation as net counts per minutes (ct/min = experimental ct/min in stimulated cultures minus the sum of background ct/min in responder and stimulator cells when cultured alone).

The statistical analysis was done by Student's *t*-test.

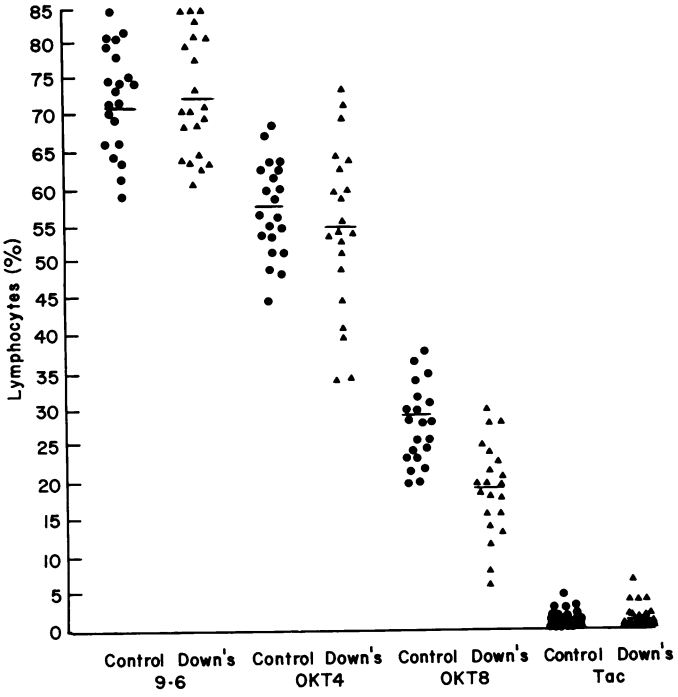

Fig. 1. Shows data of the proportions of pan-T cells (9.6^+), $OKT4^+$ (helper/inducer phenotype), $OKT8^+$ (suppressor/cytotoxic phenotype) and activated T cells (anti-Tac^+) in Down's syndrome and healthy controls.

RESULTS

Data of the analysis of lymphocyte subpopulations are shown in Fig. 1. The proportion of total T cells $9{\cdot}6^+$) and $OKT4^+$ (helper/inducer phenotype) T cells in the patient group (mean $\pm$ s.d., $9{\cdot}6^+ = 73{\cdot}1 \pm 8{\cdot}2$, $OKT4^+ = 54{\cdot}8 \pm 11{\cdot}5$) was comparable to simultaneously studied controls (mean $\pm$ s.d., $9{\cdot}6^+ = 72{\cdot}0 \pm 6{\cdot}8$, $OKT4^+ = 58{\cdot}4 \pm 6{\cdot}4$). The proportion of $OKT8^+$ T cells in the patient group (mean $\pm$ s.d., $19{\cdot}5 \pm 6{\cdot}1$) was decreased as compared to controls (mean $\pm$ s.d., $31{\cdot}4 \pm 4{\cdot}2$). This difference was statistically significant ($P < 0{\cdot}05$). Nine of 21 patients demonstrated levels of $OKT8^+$ cells below the lowest value for the simultaneously studied age and sex matched controls. Proportion of anti-Tac^+ T cells (activated T cells) in DS (mean $\pm$ s.d., $1{\cdot}7 \pm 2.1$) was similar to those of controls (mean $\pm$ s.d., $1{\cdot}3 \pm 1{\cdot}1$).

Results of the AMLR in DS and controls are shown in Fig. 2. The patients with DS had similar proliferative response in the AMLR (ct/min, mean $\pm$ s.d., $8{,}071 \pm 5{,}536$) when compared to that of simultaneously studied healthy controls (ct/min, mean $\pm$ s.d., $8{,}141 \pm 6{,}814$). None of the patients

Table 1. Allogeneic MLR between T and non-T cells from patients with Down's syndrome and controls

Responder T cells	Stimulator non-T cells	(Mean $\pm$ s.d. ct/min)
(A) Patients (21)	Controls (21)	$27{,}263 \pm 20{,}431$
(B) Controls (21)	Patients (21)	$16{,}500 \pm 6{,}893$
(C) Controls (21)	Controls (21)	$18{,}226 \pm 14{,}874$

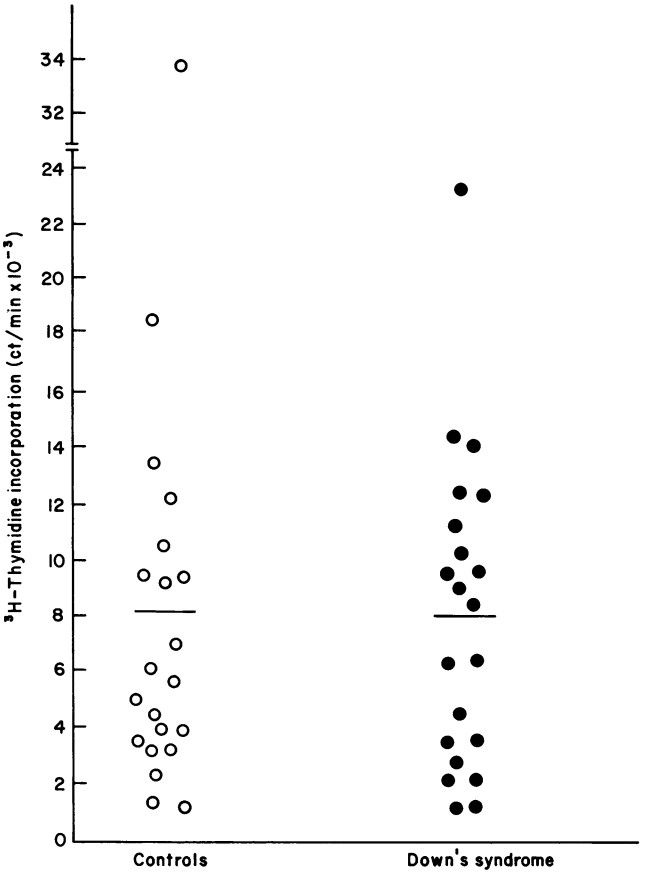

Fig. 2. AMLR in patients with Down's syndrome and simultaneously studied healthy controls. Data are presented for net ct/min.

fell outside the range for healthy controls. The control counts were also comparable in two groups (DS, 460 ± 80; controls 396 ± 94). The peak proliferative response was observed on day 6 in each patient and control group (studies were done on day 5, 6 and 7, data not shown).

The results of allogeneic MLR are shown in Table 1. Non-T cells from patients with DS had similar stimulator activity when compared to control groups. T cells from patients with DS stimulated responded more vigorously to non-T cells from healthy controls than T cells from normal when stimulated with non-T cells from allogeneic normals, however, because of high standard deviation, the differences were not significant ($P > 0{\cdot}05$).

DISCUSSION

In this study we have demonstrated normal proliferative response in the AMLR in patients with DS. Further, the proportion of OKT4$^+$ T cells were similar to those found in age and sex matched healthy controls. In contrast, the proportion of OKT8$^+$ T cells were significantly decreased ($P < 0{\cdot}05$) in patients with DS when compared to the control group. Recently it has been demonstrated that OKT4$^+$ cells are the major responder in the AMLR (Damle *et al.*, 1981; Kozak *et al.*, 1982). Therefore, the normal AMLR in DS is in agreement with the normal proportion of OKT4$^+$ in DS. Our data of AMLR do not confirm the results of Franchesi *et al.* (1981) who demonstrated deficiency of the AMLR in non-institutionalized patients with DS. These authors,

however, did not report their data for individual subjects. The discrepancies between our data and those reported by Franchesi *et al.* (1981) could be due to age differences. They had much older patients than those in the present study. The majority of our patients were below 7 years of age. In the AMLR, $OKT4^+$ T cells produce interleukin 2 (IL-2) (Palacios & Moller, 1981). We observed normal IL-2 production by all six patients with DS that were analysed for IL-2 production (data not shown).

In allogeneic MLR, patients T cells responded comparable to control group and non-T cells from patients with DS stimulated in allogeneic MLR to a similar degree that is observed in the matched control group. This observation is in agreement with Cupples & Tan (1977) and Franchesi *et al.* (1982); however, in contradiction to the higher MLR reported by Sasaki & Obara (1969).

We found normal proportion of total T cells as defined with pan-T cell monoclonal antibody (9·6). A number of investigators have reported decreased proportions of T cells as measured by rosette formation with SRBC (Frenchesi *et al.*, 1978; Levin *et al.*, 1979; Gershwin *et al.*, 1977; Ugazio *et al.*, 1977). Though the 9·6 monoclonal antibody defines receptor for SRBC on T cells, analysis with monoclonal antibody appears to be a more sensitive method. Furthermore, factors effecting binding of SRBC and T cells may not effect the reaction between antibody and T cell receptor antigen. There was no evidence of increased proportion of circulating activated T cells in DS as measured by anti-Tac antibody that defines an antigen present exclusively on activated T cells (Uchiama *et al.*, 1981).

The above data suggest that DS (at least very young patients) cannot be considered a good immunological model of accelerated ageing in man. It is, however, possible that the older patients (in 20's) with DS would show immunological defects similar to those seen in ageing humans.

REFERENCES

BOURGIO, G.R., UGAZIO, A.G., NESPOLI, L., MARCIONI, A.F., BOTELLI, A.M. & PASQUALI, F. (1975 Derangement of immunoglobulin levels, phytohemagglutinin responsiveness, and T and B cell markers in Down's syndrome at different ages. *Eur. J. Immunol.* **5,** 600.

BURGIO, F.R. & UGAZIO, A.G. (1978) Immunity in Down's syndrome. *Eur. J. Pediat.* **127,** 293.

CUPPLES, C.G. & TAN, Y.T. (1977) Effect of human interferon preparations on lymphoblastogenesis in Down's syndrome. *Nature*, **267,** 105.

DAMLE, N.K., HANSEN, J.A., GOOD, R.A. & GUPTA, S. (1981) Monoclonal antibody analysis of human T lymphocyte subpopulation exhibiting autologous mixed lymphocyte reaction. *Proc. Natl. Acad. Sci. USA.* **78,** 5098.

FIALKOW, P.J. (1970) Thyroid autoimmunity and Down's syndrome. *Ann. N.Y. Acad. Sci.* **171,** 500.

FRANCESCHI, C., LICASTRO, F., PAOLUCCI, P., MASI, M., CAVICCHI, S. & ZANOTTI, M. (1978) T and B lymphocyte subpopulations in Down's syndrome. A study of noninstitutionalized subjects. *J. Med. Def. Res.* **22,** 179.

FRANCHESCHI, C., LICASTRO, F., CHIRICOLO, M., BONETTI, F., ZANOTTI, M., FABRIS, N., MOCCHEGIANI, E., FANTINI, M.P., PAOLUCCI, P. & MASI, M. (1981) Deficiency of autologous mixed lymphocyte reactions and serum thymic factor level in Down's syndrome. *J. Immunol.* **126,** 2161.

GERSHWIN, M.E., GRINELLA, F.M., CASTLES, J.J. & TRENT, J.K.T. (1977) Immunologic characteristics of Down's syndrome. *J. Med. Def. Res.* **21,** 237.

KAMOUN, M., MARTIN, P.J., HANSEN, J.A., BROWN, A.W., SIADAK, A.W. & NOWINSKI, R.C. (1981) Identification of a human T lymphocyte surface protein associated with E. rosette receptor. *J. exp. Med.* **153,** 207.

KOZAK, R., MOODY, C.E., STAIANO-COICO, L. & WEKSLER, M.E. (1982) Lymphocyte transformation induced by autologous cells. XII. Quantitative and qualitative differences between human autologous and allogeneic reactive T lymphocytes. *J. Immunol.* **128,** 1723.

LEVIN, S., SCHLESINEGER, M., HANDZEL, Z., HAHN, T., ALTMAN, Y., CZERNOBILSKY, R. & BOSS, J. (1979) Thymic deficiency in Down's syndrome. *Pediatrics*, **63,** 80.

LOPEZ, V., OCHS, H.D., THULINE, H.C., DAVIS, S.D. & WEDGEWOOD, R.J. (1975) Defective antibody response to bacteriophage X 174 in Down's syndrome. *Am. J. Pediat.* **86,** 207.

MARTIN, G.M. (1977) In *Genetic Effects of Aging* (Ed. by E.L. Scneider). National Foundation, March of Dimes.

MOODY, C.E., INNES, J.B., STAIANO-COICO, L., INCEFY, G.S., THALER, H.T. & WEKSLER, M.E. (1981) Lymphocyte transformation induced by autologous cells. IX. The effect of age on the autologous mixed lymphocyte reaction. *Immunology*, **44,** 431.

PALACIOS, R. & MOLLER, G. (1981) HLA-DR antigens rendering resting R cells sensitive to interleukin 2 and induce production of the growth factor in the autologous mixed lymphocyte reaction. *Cell. Immunol.* **63,** 143.

REINHERZ, E.L. & SCHLOSSMAN, S.F. (1980) The differentiation and function of human T lymphocytes. *Cell*, **19,** 821.

RIGAS, D.A., ELASSER, P. & HECHT, F. (1970) Im-

paired *in vitro* response of circulating lymphocytes to phytohemagglutinin in Down's syndrome: dose and time response curves and relation to cellular immunity. *Int. Arch. Allergy Appl. Immunol.* **39,** 58.

SASAKI, M. & OBARA, Y. (1969) Hypersensitivity of lymphocytes in Down's syndrome shown by mixed leucocyte culture experiments. *Nature*, **222,** 596.

SUTNICK, A.I., LONDON, T., BLUMBERG, B.S. & GERTSLEY, J.S. (1971) Susceptibility to leukemia: immunologic factors in Down's syndrome. *J. Natl. Cancer Inst.* **47,** 923.

UCHIAMA, T., BRODER, S. & WALDMANN, T.A. (1981) A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. I. Production of anti-Tac monoclonal antibody and distribution of Tac (+) cells. *J. Immunol.* **126,** 1393.

UGAZIO, A.G., MACCARIO, R., DUSE, M. & BOURGIO, G.R. (1977) T lymphocyte deficiency in Down's syndrome. *Lancet*, **i,** 1062.

WALFORD, R.L., GESSETT, T.C., NAEIM, F., TAM, C.F., VAN LANCKER, J.L., BARNETT, E.V., CHIA, D., SPARKES, R.S., FAHEY, J.L., SPINA, C., GATTI, R.A., MEDIA, M.A., GROSSMAN, H., HIBRAUR, H. & MOTOLA, M. (1981) Immunological and biochemical studies of Down's syndrome as a model of accelerated aging. In *Immunological Aspects of Aging* (ed. by D. Segre & L. Smith.) p. 479. Marcel-Dekker, Inc., New York.

WHITTINGHAM, S., PITT, D.B., SHARMA, D.L.B. & MACKAY, I.R. (1977) Stress deficiency of the T lymphocyte system exemplified by Down's syndrome. *Lancet*, **i,** 163.