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Lymphocyte subpopulations in Down's syndrome: high percentage of circulating HNK-1$^+$, Leu 2a$^+$ cells

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SUMMARY

Peripheral blood lymphomononuclear cells (PBL) from 35 patients with Down's syndrome (DS, trisomy-21; 25 institutionalized and 10 non-institutionalized) were phenotypically characterized by means of various monoclonal antibodies. They included a high percentage of T lymphocytes with low avidity for sheep erythrocytes as well as an extremely high percentage of HNK-1$^+$ cells and of lymphocytes reacting with the OKT8 and Leu 2a antibodies. The HNK-1$^+$ cells of DS include four different subsets: (a)E$^+$, OKT3$^+$, OKT8$^-$, Leu 2a$^-$; (b) E$^+$, OKT3$^+$, OKT8$^+$, Leu 2a$^+$; (c) E$^-$, OKT3$^-$, OKT8$^-$, Leu 2a$^-$ and (d) E$^-$, OKT3$^-$, OKT8$^+$, Leu 2a$^+$. Subsets (a) and (c) are also present in PBL from karyotypically normal controls while subsets (b) and (d) have a phenotype which has not been previously reported. These findings may be related to the triple expression by trisomic cells of the receptor for interferon, which is coded by a gene located on chromosome number 21. Alternatively, the high number of 'immature' NK cells of DS, possibly identical with pre-T lymphocytes, may originate from the congenital thymic derangement associated with trisomy 21.

Keywords Down's syndrome congenital thymic derangement natural killer cells interferon pre-T lymphocytes trisomy-21

INTRODUCTION

Patients with Down's syndrome (DS, trisomy-21) have an increased susceptibility to polytopic infections (Oster, Mikkelsen & Nielsen, 1975), a high frequency of malignancies, especially leukaemia (Krivit & Good, 1957; Miller, 1970) and increased frequency of autoantibodies (Burgio *et al.*, 1965; Fialkow, 1970; Baxter *et al.*, 1975; Ugazio *et al.*, 1977).

The clinical picture of DS has been associated with a combined deficiency of antibody and cell-mediated immunity (Griffith & Silvester, 1967; Gordon, Sinha & Carlson, 1971; Burgio *et al.*, 1975; Lopez *et al.*, 1975; Whittingham *et al.*, 1977; Ugazio *et al.*, 1978; Fekete *et al.*, 1982). In particular, lymphocyte responses *in vitro* to T cell mitogens (Burgio *et al.*, 1975) and antigens (Epstein & Epstein, 1980) are weak and the percentage of circulating E rosette forming cells (E-RFC) is low (Burgio *et al.*, 1975; Levin, Nir & Hogilner, 1975; Franceschi *et al.*, 1978).

However we have previously reported that in DS, peripheral blood lymphocytes (PBL) include a high percentage of lymphocytes with low avidity for sheep erythrocytes (Burgio *et al.*, 1978, 1983).

In the present study, various subpopulations of circulating lymphocytes have been characterized in subjects with DS by using a series of monoclonal antibodies (MoAbs). Results show that

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PBL of DS include a very high percentage of cells expressing the HNK-1 antigen, a putative marker of lymphocytes with natural killer (NK) activity (Abo & Balch, 1981) together with the OKT8 and Leu 2a antigens, usually present on suppressor/killer T cells (Reinherz *et al.*, 1980; Evans *et al.*, 1981).

These HNK-1$^+$, OKT8$^+$, Leu 2a$^+$ cells include at least two subsets: one reacts with the OKT3 MoAb, a pan-T reagent (Reinherz *et al.*, 1979) and forms E rosettes, while the other does not react with the OKT3 antibody and does not form E rosettes.

MATERIALS AND METHODS

Patients. Twenty-five institutionalized subjects with karyotipically documented DS (the age ranged from 2 to 42 years; in particular seven subjects were less than 5 years old, four between 6 and 10, five between 11 and 20, nine between 21 and 42) and 25 age and sex matched controls with normal karyotype living in the same institution were included in the study. Ten children non-institutionalized (i.e. living at home) were also included in the study; the age ranged from 8 months to 7 years, three were males and seven females. Lymphocytes were isolated from heparinized peripheral blood as previously described (Burgio *et al.*, 1978).

Monocyte depletion. Monocytes were removed by plastic adherence as described (Nespoli *et al.*, 1980).

Preparation of T cell depleted and enriched fractions. T cell enriched (E$^+$) and depleted (E$^-$) fractions were obtained by rosetting PBL with 2-aminoethylisothiouronium bromide treated sheep red blood cells (AET-E-RFC) as previously described (Indiveri *et al.*, 1979; Maccario *et al.*, 1981).

'Panning' technique. Fisher 30 × 15 mm polystyrene bacteriological Petri dishes were individually coated with 2 μg of rabbit anti-mouse IgG (Flow Laboratories, Irvine, UK) diluted 1/10 with normal rabbit IgG as described by Wysocki & Sato (1978) and Reinherz *et al.*, (1981) and kept at 4°C overnight. The plates were then washed four times with 5 ml of PBS, filled with PBS supplemented with 2% FCS and maintained at 4°C for 1–4 h. The medium was removed prior to addition of the cell population. About 10–20 × 10^6 E$^-$ lymphocytes were spun and 1 ml of a 1/200 dilution of OKT8 MoAb was added to the pellet. The cells were incubated for 30 min at 4°C and then washed in PBS with 0·5% bovine serum albumin (BSA). The cell suspension was brought to a concentration of 3–4 × 10^6/ml and 0·7 ml were applied to a single anti-mouse IgG coated plate. The plates were incubated on a level surface at 4°C and after 30 min gently swirled; after a total of 60 min the non-adherent population was removed with a Pasteur pipette swirling again the dish. To recover the bound cells, after one washing the plate was filled with 10 ml PBS 2% FCS and the entire surface of the plate was flushed using a Pasteur pipette. To remove bound antibody, washed cells were treated for 1 min with saline brought to pH 3 with 2N HCl. The cells were then washed twice with medium.

E rosettes. E rosettes (E-RFC) were assessed as previously described (Burgio *et al.*, 1978).

Membrane immunofluorescence. The cells were characterized by MoAbs as previously described (Maccario *et al.*, 1983); OKT3, OKT4, OKT6, OKT8 antibodies were purchased from Ortho Pharmaceutical Corporation (Raritan, New Jersey, USA) HNK-1 (Leu 7) and Leu 2a MoAbs were purchased from Becton-Dickinson (Rutherford, New Jersey).

Double labelling with HNK-1 and Leu 2a. T cell depleted and enriched lymphocytes were first incubated with HNK-1 and stained with fluorescein labelled goat/mouse antiserum, the cell pellet was then incubated with Biotinyl Leu 2a (Becton-Dickinson) for 30 min at 0°C. After three spin washes, 0·1 ml of rhodamine labelled avidin (Vector Laboratories) was added to the cells. The tubes were maintained at 0°C for 30 min in the dark. After three further washes the slides were prepared.

RESULTS

The results obtained in the 25 institutionalized DS patients and 25 matched karyotypically normal controls confirmed previous findings of a low percentage of E-RFC in DS; nevertheless the percentages of OKT3$^+$, OKT4$^+$ and OKT6$^+$ cells were the same in the two groups (Table 1).

Table 1. Surface markers identified on PBL of 25 subjects with DS and 25 karyotypically normal controls

	E-RFC	OKT3	OKT4	OKT8	Leu 2a	OKT4 OKT8	OKT6	HNK-1
DS	56±12	72±2	43±8	45±11	52±9	85±8	4±4	37±12
Controls	72±4	74±6	43±11	25±6	23±7	65±7	2±2	16±6
P	<0·01	NS	NS	<0·001	<0·001	<0·001	NS	<0·001

Results are expressed as mean values ±s.d. of the percentage of positive cells.

The percentage of OKT8$^+$, Leu 2a$^+$ cells, putatively killer/suppressor T lymphocytes, was significantly higher in DS than in controls. In DS the percentage of circulating lymphocytes reactive with the HNK-1 MoAb, a marker of lymphocytes with NK activity, was more than twice the normal value (Table 1). Similar results were obtained in the non-institutionalized children: the percentage of OKT8$^+$ cells was 42±10 (age normal values 27±8) while the percentage of HNK-1$^+$ cells was 38±12 (age normal values 12±7).

In all subjects with DS, the sum of the percentages of OKT4$^+$ and OKT8$^+$ lymphocytes was higher than the percentage of OKT3$^+$ cells (known to include normally OKT8$^+$ and OKT4$^+$ cells), whereas in controls this sum was lower than the percentage of OKT3$^+$ cells. When PBL of DS patients and controls were simultaneously exposed to a mixture of OKT4 and OKT8 MoAbs, the percentage of positive cells was again lower than the percentage of OKT3$^+$ lymphocytes in controls but higher in DS thus suggesting that PBL from DS include a subset of OKT3 negative lymphocytes reacting with the OKT4 or the OKT8 antibodies. To test between these two possibilities, PBL of patients with DS and controls were first depleted of adherent cells and then separated in E-RFC depleted (E$^-$) and E-RFC enriched (E$^+$) fractions. The E$^+$ and E$^-$ fractions were then characterized by reactivity with the OKT3, OKT8, Leu 2a and HNK-1 MoAbs (Table 2). After E-RFC depletion, PBL from both DS and controls were substantially devoid of OKT3$^+$ cells; however PBL from controls were also devoid of OKT8$^+$ lymphocytes while PBL from DS included a high percentage of OKT8$^+$ lymphocytes. Similar results were obtained with the Leu 2a MoAb. In the E$^-$ fractions, the percentage of HNK-1$^+$ cells was higher in DS than in controls.

Table 2. Surface markers identified on E$^-$ and E$^+$ fractions in 10 subjects with DS and 10 karyotypically normal controls

	E$^-$				E$^+$			
	OKT3	OKT8	Leu 2a	HNK-1	OKT3	OKT8	Leu 2a	HNK-1
DS	6±4	33±11	31±9	48±7	90	44±5	43±8	46±13
Controls	3±2	2±2	0	27±7	90	30±3	31±4	12±7
P	NS	<0·001	<0·001	<0·001	NS	<0·001	<0·001	<0·001

Results are expressed as mean values ±s.d. of the percentage of positive cells.

In the E$^+$ fraction, the percentage of OKT3$^+$ cells was 90% in the two groups and the percentage of both OKT8$^+$, Leu 2a$^+$ and NHK-1$^+$ cells was higher in DS than in controls. As reported in Table 3, double labelling with Leu 2a and HNK-1 MoAbs of E$^-$ and E$^+$ fractions showed that in DS almost all Leu 2a$^+$ cells in the E$^-$ fraction were also HNK-1$^+$ while in controls E$^-$ cells were substantially devoid of Leu 2a$^+$/HNK-1$^+$ cells; on the contrary the percentage of HNK-1$^+$/Leu 2a$^-$ cells was the same in the two groups. In the E$^+$ fraction from DS, most HNK-1$^+$ cells were also Leu 2a$^+$ but a subset of HNK-1$^+$ cells did not react with the Leu 2a MoAb; the E$^+$ fraction from controls only included HNK-1$^+$/Leu 2a$^-$ cells. In three separate experiments the OKT8$^+$, Leu 2a$^+$

Table 3. Double labelling with Leu 2a and HNK-1 MoAbs on E^- and E^+ fractions in eight subjects with DS and eight karyotypically normal controls

	E^-			E^+		
	Leu $2a^+$	HNK-1^+	HNK-$1^+/2a^+$	Leu $2a^+$	HNK-1^+	HNK-1^+/Leu 2a
DS	3 ± 1	26 ± 8	27 ± 6	16 ± 6	15 ± 7	27 ± 11
Controls	2 ± 1	27 ± 5	1 ± 1	28 ± 6	10 ± 6	3 ± 2

Results are expressed as mean values $\pm$s.d. of the percentage of positive cells.

cells present in the E^- fraction were enriched by 'panning' with the Leu 2a MoAb: in all experiments, more than 80% of the cells were $OKT8^+$ and HNK-1^+ with less than 1% of contaminating $OKT3^+$ lymphocytes.

DISCUSSION

The present study shows that PBL of patients with DS include a low percentage of E-RFC, a normal number of $OKT3^+$ cells, a high percentage of $OKT8^+$, Leu $2a^+$ lymphocytes and an extremely high number of HNK-1^+ cells. In fact the E^- fraction of PBL from subjects with DS was found to include a sizeable subset of $OKT3^-$ cells not present in PBL from controls and characterized by the simultaneous expression of the OKT8, Leu 2a and HNK-1 antigens. As HNK-1^+ cells are known to express receptors with low avidity for sheep erythrocytes (West, Boozer & Herberman, 1978) the presence of this subset may justify previous finding of a high percentage of lymphocytes with low avidity for sheep erythrocytes in DS (Burgio *et al.*, 1978); furthermore these cells are $OKT8^+$ and $OKT3^-$, thus accounting for the finding that the combined percentages of $OKT8^+$ and $OKT4^+$ lymphocytes exceeds that of $OKT3^+$ cells in DS but not in normal subjects. Derangements of the OKT8 subset in DS have been reported in non-institutionalized children with DS by Gupta *et al.* (1983); however, at variance with the present results, Gupta *et al.* (1983) have found low rather than high numbers of $OKT8^+$ cells. The reasons for this discrepancy remain to be established. The percentage of HNK-1^+ cells was higher in DS than in controls also within the E^+, fraction. HNK-1^+ cells include $OKT8^-$ cells also present in controls as well as $OKT8^+$ cells usually not found in karyotypically normal subjects. On the whole, the present data show that circulating mononuclear cells from DS include at least four subsets of HNK-1^+ cells: (a) E^+, $OKT3^+$, $OKT8^-$, Leu $2a^-$; (b) E^+, $OKT3^+$, $OKT8^+$, Leu $2a^+$; (c) E^-, $OKT3^-$, $OKT8^-$, Leu $2a^-$ and (d) E^-, $OKT3^-$, $OKT8^+$, Leu $2a^+$. Subsets (a) and (c) are also present in PBL from normal donors while subset (d) has a surface phenotype which has not been previously reported and subset (b) has a membrane phenotype normally found only among bone marrow lymphocytes. The mechanism underlying the high number of circulating HNK-1^+ cells in DS as well as the appearance of unusual subsets of HNK-1^+ lymphocytes are presently obscure.

Interferon (IFN) is known to increase NK activity (Herberman & Holden, 1978; Trinchieri & Santoli, 1978; Silva, Bonovida & Targan, 1980; Targan & Dorey, 1980): the gene responsible for the cellular response to IFN has been mapped on chromosome 21 (Tan, 1975) and the triple representation of this gene in DS results in an increased susceptibility to IFN of trisomic cells (Epstein & Epstein, 1980; Epstein, Lee & Epstein, 1980; Mogensen, Vignaux & Gresser, 1982). It may be hypothesized that this hypersensitivity of trisomic cells to IFN is also the ultimate cause of the high number of NHK-1^+ cells in DS. An alternative explanation is that NK cells are massively produced and/or released because of the high frequency of infections; however in the present study, the percentage of HNK-1^+ cells was not increased in karyotypically normal controls living in the same institution and therefore exposed to the same microbial load as DS subjects. Furthermore subjects with DS did not show an age-dependent increase of the percentage of HNK-1^+ cells although adults with DS have certainly experienced a higher number of infections than children.

Abo *et al.* (1983) have suggested on the basis of ontogenetic and functional studies that the HNK-1$^+$, OKT3$^+$ cells are in fact immature lymphocytes with low NK activity. The subset (d) of HNK-1$^+$, E$^-$, OKT3$^-$, OKT8$^+$, Leu 2a$^+$ cells shares several phenotypic features with a recently described subset of neonatal lymphocytes (Maccario *et al.*, 1983; Vitiello *et al.*, 1983) characterized by the phenotype HNK-1$^-$, E$^-$, OKT3$^-$, OKT8$^+$, Leu 2a$^+$ displaying NK activity; this neonatal subset also expresses a membrane receptor for the peanut agglutinin (PNA), a marker of immature cells (London, Berrih & Bach, 1978; Reisner *et al.*, 1979). Furthermore, although this subset does not react with the HNK-1 MoAb, it reacts with the MoAb B73·1 specific for NK cells (Perussia *et al.*, 1983a, 1983b). Thus, the subset of HNK-1$^+$, OKT3$^-$, OKT8$^+$, Leu 2a$^+$ cells found in DS may represent an early, immature stage of differentiation of NK cells: in fact, although the number of HNK-1$^+$ cells is very high, NK activity in DS is not significantly different from that of controls (Nurmi *et al.*, 1982).

Roder, Karre & Kiessling (1981) have suggested that both NK cells and pre-T lymphocytes originate at least in part from a common progenitor so that when the thymus is absent or hypoplasic the impaired differentiation along the thymic axis leads to a preferential differentiation of the common progenitor to NK cell. This may be the mechanism leading to the observed high NK activity of the athymic nude mouse (Kiessling *et al.*, 1976; Warner, Woodruff & Burton, 1977; Minato *et al.*, 1979). Patients with DS are known to suffer from a congenital dysplasia of the thymus, manifested both morphologically (Benda & Strassman, 1965; Levin *et al.*, 1979) and by an impaired production of thymic hormonal factors (Handzel *et al.*, 1979; Duse *et al.*, 1980). The presence in DS of a large percentage of NK cells together with the occurrence of a severe thymic derangement further supports the hypothesis that NK cells are under the indirect control of the thymus.

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REFERENCES

ABO, T. & BALCH, C.M. (1981) A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). *J. Immunol.* **127,** 1027.

ABO, T., MILLER, C.A., GARTLAND, G.L. & BALCH, C.M. (1983) Differentiation stages of human natural killer cells in lymphoid tissues from fetal to adult life. *J. exp. Med.* **157,** 273.

BAXTER, R.G., LARKINS, R.G., MARTIN, F.I.R., HEYMA, P., MYLEX, K. & RYAN, L. (1975) Down syndrome and thyroid function in adults. *Lancet*, **ii,** 794.

BENDA, C.E. & STRASSMAN, G.S. (1965) The thymus in mongolism. *J. Ment. Defic. Res.* **9,** 109.

BURGIO, G.R., SEVERI, F., ROSSONI, R. & VACCARO, R. (1965) Mongolism and thyroid autoimmunity. *Lancet*, **i,** 166.

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

BURGIO, G.R., LANZAVECCHIA, A., MACCARIO, R., VITIELLO, A., PLEBANI, A. & UGAZIO, A.G. (1978) Immunodeficiency in Down's syndrome: T lymphocyte subset imbalance in trisomic children. *Clin. exp. Immunol.* **33,** 298.

BURGIO, G.R., UGAZIO, A.G., NESPOLI, L. & MACCARIO, R. (1983) Down syndrome: a model of immunodeficiency. *Nation. Found. March of Dimes* (In press.)

DUSE, M., BRUGO, M.A., MARTINI, A., TASSI, C., FERRARIO, C. & UGAZIO, A.G. (1980) Immunodeficiency in Down's syndrome: low levels of serum thymic factor in trisomic children. *Thymus*, **2,** 127.

EPSTEIN, L.B. & EPSTEIN, C.J. (1980) T lymphocyte function and sensitivity to interferon in trisomy-21. *Cell. Immunol.* **51,** 303.

EPSTEIN, L.B., LEE, S.H.S. & EPSTEIN, C.J. (1980) Enhanced sensitivity of trisomy-21 monocytes to the maturation-inhibiting effect of interferon. *Cell Immunol.* **50,** 191.

EVANS, R.L., WALL, D.W., PLATSONCAS, C.D., SIEGAL, F.P., FIKRIG, S.M., TESTA, C.M. & GOOD, R.A. (1981) Thymus dependent membrane antigens in man: inhibition of cell mediated lympholysis by monoclonal antibody to the TH_2 antigens. *Proc. Natl. Acad. Sci. USA.* **78,** 544.

FEKETE, G., KULCSAR, G., DAUN, P., NASZ, I. SCHULER, D. & DOBOS, M. (1982) Immunological and virological investigations in Down's syndrome. *Eur. J. Paediatr.* **138,** 59.

FIALKOW, P.J. (1979) Thyroid autoimmunity and Down's syndrome. *Ann. N.Y. ACAD. Sci.* **197,** 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 on non-institutionalized subjects. *J. Ment. Defic. Res.* **22,** 179.

GORDON, M.C., SINHA, S.K. & CARLSON, S.D. (1971) Antibody responses to influenza vaccine in patients with Down's syndrome. *Am J. Ment. Defic.* **75,** 391.

GRIFFITH, A.W. & SILVESTER, P.E. (1967) Mongols and non-mongols compared in their response to active tetanus immunization. *J. Ment. Defic. Res.* **11,** 263.

GUPTA, S., FIKRIG, S.M., MARIANO, E. & QUAZI, Q. (1983) Monoclonal antibody defined T cell subsets and autologous mixed lymphocyte reaction in Down's syndrome. *Clin. exp. Immunol.* **53,** 25.

HANDZEL, Z.T., DOLFIN, Z., LEVIN, S., ALTMAN, Y., HAHN, T., TRAININ, N. & GADOT, N. (1979) Effect of thymic humoral factor on cellular immune function of normal children and of pediatric patients with ataxia telangiectasia and Down's syndrome. *Pediatr. Res.* **13,** 803.

HERBERMAN, R.B. & HOLDEN, H.R. (1978) Natural cell-mediated immunity. *Adv. Cancer Res.* **27,** 305.

INDIVERI, F., PELLEGRINO, M.A., MOLINARO, G.A., QUARANTA, V. & FERRONE S. (1979) Rosetting of human T lymphocytes with goat red cells: effect of treatment with 2-aminoethylisothiouronium bromide (AET) and comparison with treated sheep red blood cells. *J. Immunol. Meth.* **30,** 317.

KIESSLING, R., PETRANY, G., KLEIN, G. & WIGZELL, H. (1976) Non-T cell resistance against a mouse moloney lymphoma. *Int. J. Cancer*, **17,** 1.

KRIVIT, W. & GOOD, R.A. (1957) Simultaneous occurrence of mongolism and leukemia. *Am. J. Dis. Child.* **94,** 289.

LEVIN, S., NIR, E. & HOGILNER, B.M. (1975) T system immune deficiency in Down's syndrome. *Pediatrics*, **56,** 123.

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

LONDON, J., BERRIH, S. & BACH, J.F. (1978) Peanut agglutinin. I. A new tool for studying lymphocyte subpopulations. *J. Immunol.* **121,** 438.

LOPEZ, V., OCHS, H.D., THULINE, H.C., DAVIS, S.D. & WEDGWOOD, R.J. (1975) Defective antibody response to bacteriophage Φ X174 in Down's syndrome. *J. Pediatr.* **86,** 207.

MACCARIO, R., FERRARI, F.A., SIENA, S., VITIELLO, A., MARTINI, A., SICCARDI, A.G. & UGAZIO, A.G. (1981) Receptor for peanut agglutinin on a high percentage of human cord blood lymphocytes; phenotype characterization of peanut positive cells. *Thymus*, **2,** 239.

MACCARIO, R., NESPOLI, L., MINGRAT, G., VITIELLO, A., UGAZIO, A.G. & BURGIO, G.R. (1983) Lymphocyte subpopulations in the neonate: identification of an immature subset of OKT8-positive, OKT3-negative cells. *J. Immunol.* **130,** 1129.

MILLER, R.W. (1970) Neoplasia and Down's syndrome. *Ann. N.Y. Acad. Sci.* **171,** 637.

MINATO, N., BLOOM, B.R., JONES, C., HOLLAND, J. & REID, L.M. (1979) Mechanism of rejection of virus persistently infected tumor cells by athymic nude mice. *J. exp. Med.* **149,** 1117.

MOGENSEN, K.E., VIGNAUX, F. & GRESSER, I. (1982) Enhanced expression of cellular receptors for human interferon-α on peripheral lymphocytes from patients with Down's syndrome. *FEBS Lett.* **140,** 285.

NESPOLI, L., VITIELLO, A., MACCARIO, R., LANZAVECCHIA, A. & UGAZIO, A.G. (1980) Activation of human peripheral blood lymphocytes: effect of concanavalin A and lypopolysaccaride on *in vitro* synthesis of DNA and immunoglobulins. *Scand. J. Immunol.* **12,** 165.

NURMI, R., HUTTUNEN, K., LASSILA, O., HENTTONEN, M., SAKKINEN, A., LINNA, S.L. & TIILIKAINEN, A. (1982) Natural killer cell function in trisomy-21 (Down's syndrome). *Clin. exp. Immunol.* **47,** 735.

OSTER, J. MIKKELSEN, M. & NIELSEN, A. (1975) Mortality and life-table in Down's syndrome. *Acta Paediatr. Scand.* **64,** 322.

PERUSSIA, B., STARR, S., ABRAHAM, S., FANNING, V. & TRINCHIERI, G. (1983a) Human natural killer cells analyzed by B73,1, monoclonal antibody blocking Fc receptor functions. I. Characterization of the lymphocyte subset reactive with B73,1. *J. Immunol.* **130,** 2133.

PERUSSIA, B., ACUTO, O., TERHORST, C., FAUST, J., LAZARUS, R., FANNING, V. & TRINCHIERI, G. (1983b) Human natural killer cells analyzed by B73.1. A monoclonal antibody blocking Fc receptor functions. II. Studies of B73.1 antibody-antigen interaction on the lymphocyte membrane. *J. Immunol.* **130,** 2142.

REINHERZ, E.L., KUNG, P.C., GOLDSTEIN, G. & SCHLOSSMAN, S.F. (1979) Separation of functional subsets of human T cells by a monoclonal antibody. *Proc. Natl. Acad. Sci. USA.* **76,** 4061.

REINHERZ, E.L., KUNG, P.C., GOLDSTEIN, G. & SCHLOSSMAN, S.F. (1980) A monoclonal antibody reactive with the cytotoxic suppressor T cell subset previously defined by on heteroantiserum termed TH_2. *J. Immunol.* **124,** 1301.

REINHERZ, E.L., PENTA, A.C., HOSSEY, R.E. & SCHLOSSMAN S.F. (1981) A rapid method for separating fucntionally intact human T lymphocytes with monoclonal antibodies. *Clin. Immunol. Immunopathol.* **21,** 257.

REISNER, Y., BINIAMINOV, M., ROSENTAL, E., SHARON, N. & RAMOT, B. (1979) Interaction of peanut agglutinin with normal human lymphocytes with leukemic cells. *Proc. Natl. Acad. Sci. USA.* **76,** 447.

RODER, J.C., KARRE, K. & KIESSLING, R. (1981) Natural killer cells. *Progr. Allergy*, **28,** 66.

SILVA, A., BONOVIDA, B. & TARGAN, S. Mode of action of interferon mediated modulation of natural killer cytotoxic activity: recruitment of pre-NK cells and enhanced kinetics of lysis. *J. Immunol.* **125,** 479.

TAN, Y.H. (1975) Chromosome-21-dosage effect on inducibility of anti-viral gene(s). *Nature*, **253,** 280.

TARGAN, S. & DOREY, F. (1980) Interferon activation of 'pre-spontaneous killer' (pre-k) cells and alteration in kinetics of lysis of both 'pre-SK' and active SK cells. *J. Immunol.* **124,** 2157.

TRINCHIERI, G. & SANTOLI, D. (1978) Anti-viral activity induced by colturing lymphocytes with tumor-derived or virus transformed cells. Enhancement of human natural killer cell activity by interferon and antagonist inhibition of susceptibility of target cells to lysis. *J. exp. Med.* **147,** 1314.

UGAZIO, A.G., JAYAKAR, S., MARCIONI, A.F., DUSE, M., MONAFO, V., PASQUALI, F. & BURGIO, G.R. (1977) Immunodeficiency in Down's syndrome: relationship between presence of human thyroglobulin antibodies and HBs Ag carrier status. *Eur. J. Pediatr.* **126,** 139.

UGAZIO, A.G., LANZAVECCHIA, A., JAYAKAR, S., PLEBANI, A. DUSE, M. & BURGIO, G.R. (1978) Immunodeficiency in Down's syndrome. Titers of 'natural' antibodies to *E. Coli* and rabbit erythrocytes at different ages. *Acta Paediatr. Scand.* **67,** 705.

VITIELLO, A., MACCARIO, R., MONTAGNA, D., PORTA, F.A., ALBERINI, C.M., MINGRAT, G., ASTALDI, G.C.B., NESPOLI, L. & UGAZIO, A.G. (1983) Lymphocyte subpopulations in the neonate: a subset of HNK-1$^-$, OKT3$^-$, OKT8$^+$ lymphocytes displays NK activity. *Cell. Immunol.* (In press.)

WARNER, N.L., WOODRUFF, M.F. & BURTON, R.C. (1977) Inhibition of the growth of lymphoid tumor in syngeneic, athymic (nude) mice. *Int. J. Cancer,* **20,** 146.

WEST, W.H., BOOZER, R.B. & HERBERMAN, R.B. (1978) Low affinity E-rosette formation by the human K cell. *J. Immunol.* **120,** 90.

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

WYSOCKI, L.J. & SATO, V.L. (1978) 'Panning' for lymphocytes: method for cell selection. *Proc. Natl. Acad. Sci. USA.* **75,** 2844.