Eur J Pediatr (1988) 148:53–57

European Journal of
Pediatrics

Immunology

Cell-mediated cytotoxicity in Down syndrome: impairment of allogeneic mixed lymphocyte reaction, NK and NK-like activities

D. Montagna[1], R. Maccario[1], A. G. Ugazio[2], L. Nespoli[1], E. Pedroni[1], P. Faggiano[1], and G. R. Burgio[1]

[1]Department of Paediatrics, University of Pavia, Policlinico San Matteo, Piazzale Golgi 2, I-27100 Pavia, Italy
[2]Department of Paediatrics, University of Brescia, Brescia, Italy

Abstract. Peripheral blood mononuclear cells (PBMC) from 16 non-institutionalized patients with Down syndrome (DS) were studied with various monoclonal antibodies and analysed for natural killer (NK), and NK-like activity. Lymphocyte proliferation and cytotoxic T-lymphocyte (CTL) cytotoxicity generated in mixed lymphocyte culture (MLC) were also evaluated in 11 DS patients. Phenotypic characterization of PBMC from DS subjects confirms our previous findings of high numbers of CD8+ lymphocytes and HNK−1+, and CD16+ cells. Lymphocyte proliferation and CTL cytotoxicity generated in MLC were low or absent in most patients. NK activity was low in almost all DS patients, while NK-like cytotoxicity generated in MLC was normal in the majority and did not correlate with NK activity from unstimulated PBMC.

Key words: Down syndrome – Immunodeficiency – Mixed lymphocyte reaction – NK activity

Introduction

Considerable clinical and laboratory evidence has demonstrated a combined deficiency of cell-mediated and humoral immunity in patients with Down syndrome (DS) [2, 3, 5, 8, 9, 11, 15, 16, 20, 21, 32, 35, 46, 55, 58, 61, 62, 64]. In particular, abnormalities of cell-mediated immunity include morphological alterations of the thymus, decreased T-lymphocyte number and responses to mitogens and antigens as well as depressed activity of different thymus-dependent hormonal-like activities [4, 10, 13, 17, 22, 29, 33, 34, 43, 49].

We have previously reported that in DS, peripheral blood mononuclear cells (PBMC) include high numbers of CD8+ lymphocytes and HNK−1+ cells [37]. In the present study, lymphocyte proliferation, cytotoxic T lymphocyte (CTL) activity, natural killer (NK-)like activity generated in allogeneic mixed lymphocyte cultures (MLC) and NK activity were evaluated in non-institutionalized trisomic and karyotypically normal control subjects.

Offprint requests to: G. R. Burgio

Abbreviations: BSA = bovine serum albumin; DS = Down syndrome; CTL = cytotoxic T lymphocytes; FCS = fetal calf serum; MLC = mixed lymphocyte culture; NK = natural killer; PBMC = peripheral blood mononuclear cells; PBS = phosphate-buffered saline; PHA = phytohaemagglutinin

Patients and methods

Patients

Peripheral blood samples were obtained from 16 non-institutionalized patients with trisomy 21. Their ages ranged from 4 to 15 years, with 2 subjects less than 5 years old, 5 between 5 and 10 years, and 9 between 10 and 15 years. Sixteen age-matched karyotypically normal subjects served as controls.

All subjects were free of infection at the time of the study.

Cell preparation

PBMC were isolated by means of a Ficoll-Hypaque density gradient. The cells were resuspended in RPMI 1640 medium supplemented with 2 m*M* glutamine, 50 μg/ml gentamicin and 5×10^{-5} M 2-mercaptoethanol (complete medium).

Membrane immunofluorescence with monoclonal antibodies

Leu 7 (HNK-1) and Leu 11a (CD16) were purchased from Becton Dickinson (Mountain View, Calif., USA); OKT3 (CD3), OKT4 (CD4) and OKT8 (CD8) were purchased from Ortho Pharmaceutica (Raritan, NJ, USA). Mononuclear cells (3×10^5) were resuspended in 0.2 ml phosphate-buffered saline (PBS) supplemented with 0.01M sodium azide and 0.5% bovine serum albumin (BSA) and incubated with the optimal dilution of each antibody for 30 min in an ice bath. The cell pellet was then washed twice and resuspended in 0.3 ml PBS containing the second antibody, a fluorescein-labelled F(ab')2 goat/mouse IgG (Techno-Genetics; Englewood Cliffs, NJ, USA) diluted 1/40 (v/v). The suspension was incubated for 30 min in an ice bath, the cell pellet washed resuspended in a small volume of glycerol-PBS and counted using a Leitz Orthoplan microscope equipped for epifluorescence and phase contrast. Two hundred lymphocytes were counted. Monocytes were excluded on the basis of morphological criteria.

NK assay

Natural killing was assessed using K562, an erythroleukaemia cell line, as target cells. K562 cells were maintained by serial passage in complete medium supplemented with 10% fetal calf serum (FCS). The cytotoxicity assay was performed in U-shaped wells of microtitre plates. Target cells (2×10^6) were labelled with 100 μCi of sodium ^{51}chromate solution (New England Nuclear, HANA Media inc., Berkely, Calif., USA)

for 60 min at 37°C in 0.2 ml RPMI medium supplemented with 10% FCS. The cells were then washed four times and resuspended in complete medium supplemented with 10% FCS. A volume of 0.1 ml containing 5×10^3 cells was introduced into each well. PBMC were used as effector cells. Various concentrations of effector cells in 0.1 ml complete medium supplemented with 10% FCS were added to the wells so as to achieve an effector to target ratio of 100:1, 30:1 and 10:1. The plates were centrifuged for 4 min at 200 *g* and incubated for 4 h at 37°C. After incubation the plates were centrifuged for 8 min at 200 *g* and 100 µl of the supernatant from each well was collected and counted for 1 min in a gamma counter. Maximal release was determined by freezing and thawing the target cells. The percentage of isotope released was calculated by the formula:

$$\% \text{ release} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100$$

Spontaneous release from the target cells was always less than 15%.

MLC

To measure cell proliferation generated in ony-way MLC, 10^5 responder PBMC were cultured in triplicate in U-shaped wells of microtitre plates in complete medium supplemented with 10% human serum with 10^5 irradiated (3000 R) allogeneic human PBMC as stimulators. Cultures were incubated at 37°C in a humidified 5% CO_2 atmosphere. After 6 days incubation, the cultures were labelled for 21 h with 0.5 µCi/well of ^{3}HTdR (methyl-^{3}H thymidine, 2 Ci/mmol, Amsterdam inc., Buckinghamshire, United Kingdom) and then harvested by the standard procedure. The results were expressed as mean counts of triplicate cultures. To measure NK-like and CTL activity generated in MCL, responder PBMC were cultured in 24-well plates at a concentration of 10^6/ml. Irradiated allogeneic stimulator cells were added at the same concentration in a total volume of 2 ml. After 7 days incubation, cells were recovered and NK-like and CTL activity was measured.

Phytohaemagglutinin-induced lymphoblast activation

Allogeneic human PBMC from the stimulator population were cultured in 24-well plates at a concentration of 10^6/ml in complete medium supplemented with 10% FCS. PHA-M (Gibco, Paisley, Scotland) was added at a concentration of 1% (vol/vol). After 72 h incubation the cells were recovered and labelled with 100 µCi sodium ^{51}chromate solution as described above.

CTL-mediated cytotoxicity and NK-like activity

Seven-day MLC cell populations were tested for cytolytic activity using PHA-induced lymphoblasts derived from the stimulator population for CTL activity or K562 cell line for NK-like activity under the same conditions as described for the NK assay. The specificity of CTL activity was tested by utilizing additional allogeneic mononuclear target populations unrelated to the PBMC used in the initial sensitization phase of MLC.

Statistical analysis

Student's *t*-test for unpaired sample was used to analyse the data. Values below $P < 0.05$ were considered significant.

Table 1. Surface markers identified on PBMC from 16 subjects with DS and 16 karyotypically normal controls. Results are expressed as mean values ± SD of the percentage of positive cells; NS = not significant

	HNK−1	CD16	CD3	CD4	CD8
DS	35 ± 12	30 ± 9	71 ± 6	45 ± 7	41 ± 12
Controls	16 ± 7	23 ± 5	75 ± 6	43 ± 9	26 ± 4
P	< 0.001	< 0.01	NS	NS	< 0.001

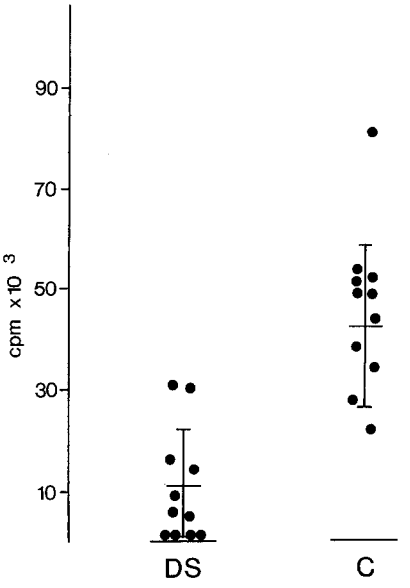

Fig. 1. Proliferative response in MLC of PBMC from 11 DS subjects and from 11 karyotypically normal controls (*C*). *Vertical bars:* 1 SD of the mean. Results are expressed as cpm/culture

Results

Table 1 shows that peripheral blood lymphocytes from the 16 subjects with DS included a higher number of HNK−1+ and CD8+ cells than those from the 16 karyotypically normal controls. The presence of a high proportion of lymphocytes with NK phenotype was confirmed by the finding of a high percentage of CD16+ cells. The proportions of CD3+ and CD4+ lymphocytes were similar to those of controls.

As shown in Fig. 1 the proliferative response in allogeneic MLC in 11 subjects with DS was absent or significantly lower as compared with 11 karyotypically normal controls ($P < 0.001$).

Evaluation of CTL activity generated in allogeneic MLC is depicted in Fig. 2. CTL activity was depressed at different effector to target ratios in 6–8 of the 11 DS subjects tested. CTL generated in MLC were specific for the sensitizing population and did not kill unrelated peripheral blood mononuclear target cells (data not shown). Mean values of CTL activity were significantly lower in 11 DS subjects than in 11 controls at effector to target ratios of 30:1 ($P < 0.001$) and 10:1 ($P < 0.005$) but not significantly different at an effector to target ratio of 100:1.

The NK activity using three different effector to target ratios is reported in Fig. 3 and shows that, in spite of elevated numbers of HNK−1+ and CD16+ lymphocytes, NK activity was significantly lower in DS than in karyotypically normal controls ($P < 0.001$).

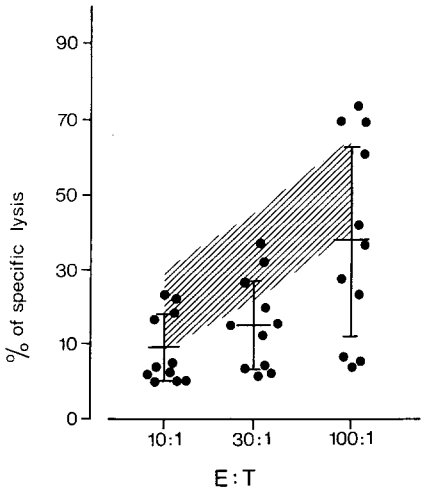

Fig. 2. CTL activity of PBMC of 11 subjects with DS (●). Effector to target ratio, 100:1, 30:1; 10:1. *Vertical bars:* 1SD of the DS mean. *Shaded area* 1SD of the karyotypically normal control mean

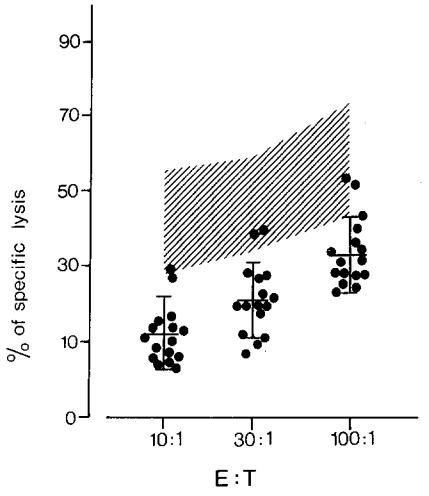

Fig. 3. NK activity of PBMC from 16 subjects with DS (●). Effector to target ratio, 100:1, 30:1; 10:1. *Vertical bars:* 1SD of the DS mean. *Shaded area* 1SD of the karyotypically normal control mean

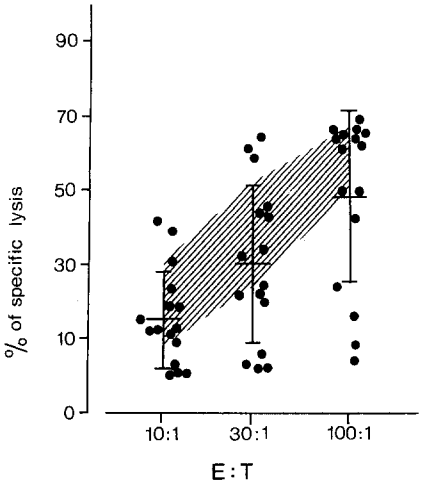

Fig. 4. NK-like activity of PBMC for 16 subjects with DS (●). Effector to target ratio, 100:1; 30:1; 10:1. *Vertical bars:* 1SD of the DS mean. *Shaded area* 1SD of the karyotypically normal control mean

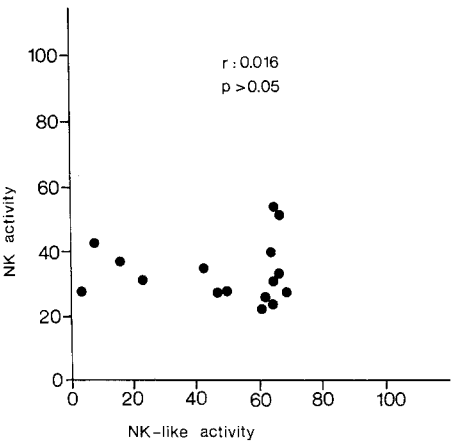

Fig. 5. Correlation between NK activity and NK-like activity of PBMC of 16 subjects with DS. Results are expressed as percentage of specific lysis. Effector to target ratio, 100:1

Evaluation of NK-like activity generated in allogeneic MLC showed heterogeneous results at different effector to target ratios. Low levels of cytotoxicity were found in 4–7 of 16 subjects with DS (Fig. 4). Mean values of NK-like activity in DS subjects were not significantly different as compared with normal controls. NK activity in fresh PBMC was not correlated with the NK-like activity generated in MLC (Fig. 5). Furthermore, NK-like activity was not correlated either with the proliferative response in allogeneic MLC or with CTL activity (data not shown).

Discussion

The present study shows that NK activity and MLC-induced lymphocyte proliferation are low in DS patients compared with karyotypically normal age-matched controls. The NK-like and CTL-activity generated in MLC varied from very low to normal in DS subjects. Phenotypic characterization of PBMC subpopulations confirmed our previous findings [37] of high numbers of CD8+, HNK−1+ and CD16+ cells in DS.

Evaluation of specific CTL activity generated in MLC demonstrated low or absent CTL activity in 7 of the 11DS subjects tested. This pattern of response was not related to MLC-induced proliferation, which was depressed in 9 DS subjects tested and is in keeping with previous observations of CTL activity in cultures with negative MLC-induced proliferation [6, 7, 42, 65].

Our data on the low proliferative response of DS lymphocytes in MLC confirm the data of Walford et al. [62], although they are not in agreement with the normal or even higher values of proliferation in MLC reported by other groups [17, 22, 54]. Reasons for this discrepancy remain to be established.

The low proliferative response of DS lymphocytes in MLC and low CTL activity in more than half of the trisomic children tested demonstrate that a T-cell defect is an integral feature of DS.

The finding of low levels of NK activity in spite of elevated numbers of lymphocytes with an NK phenotype can be explained by the previous finding that in DS the majority of HNK−1+ cells are also CD3+ [37]. In fact, it has been re-

ported [1] that HNK−1+ CD3+ lymphocytes have low NK activity as compared with HNK−1+ CD11+ cells, which includes the majority of HNK−1+ cells in normal subjects. Abo et al. [1] reported that virtually all HNK−1+ bone marrow cells express the CD3 antigen and proposed that these lymphocyte subsets represent a functionally immature stage in NK-cell differentiation. According to this hypothesis the majority of circulating HNK−1+ lymphocytes of DS subjects are immature cells with low NK activity.

Much evidence suggests that NK cells are implicated in tumour immunity, resistance to viral and microbial infections as well as the regulation of haematopoiesis and B-cell activity [23–25, 27, 45, 51, 54]. Furthermore, studies with both murine and human tumour cells suggest that different subpopulations of tumour cytotoxic cells are activated by allostimulation [19, 26, 47, 57]; these effector cells include both T-lymphocytes and NK-like cells. Allostimulation induces tumour reactive cells by various mechanisms, including the release of lymphokines and the expression of cross-reactive alloantigens on tumour targets [48].

The study of NK and NK-like activities and CTL cytotoxicity generated in MLC is important in trisomic patients, given the increased incidence of infections and malignancies and the defective regulation of granulopoiesis in DS [11, 30, 32, 39, 46, 55].

Attempts to correlate the non-specific effector activity generated in MLC (NK-like activity) with NK activity of unstimulated PBMC or with specific CTL activity have led to conflicting results [36, 40, 50, 56]. Some evidence suggests that NK-like activity is mediated by MLC-induced T cells [40], or that NK-like activity is mediated by a subpopulation of circulating NK cells [50, 56]. Our data show no correlation between NK activity and NK-like activity in DS and NK activity was low in spite of a very high percentage of cells expressing HNK−1, CD16 antigens. One explanation is that NK activity is low in DS because the majority of NK cells are immature (HNK−1+, CD3+). These cells can be stimulated in vitro during MLC to differentiate into mature NK cells expressing normal levels of NK-like cytotoxicity. In fact, it is well known that the IFRec gene, coding for the species-specific response to interferon, is localized on the human chromosome 21 and that trisomic cells including fibroblasts, monocytes, lymphocytes and NK cells have enhanced sensitivity to the effect of interferon [12, 14, 18, 41, 43, 44, 53, 59, 60, 63]. During MLC various lymphokines, including interferon, are produced and the enhanced sensitivity of NK cells to interferon may contribute to their maturation, resulting in normal NK-like activity in the majority of DS subjects. An alternative hypothesis is that NK lymphocytes and precursors of NK-like cells belong to different subpopulations and PBMC from the majority of DS subjects include a normal number of the latter population.

The relationship between T and NK cells is still a matter of debate [31, 45, 66]. However, some NK cell subsets show common steps of differentiation with T-cells [28, 38, 45]. In DS the defect in NK activity is associated with an abnormally high number of circulating HNK−1+ CD3+ lymphocytes and with impairment of T lymphocyte function. It is conceivable that many immunological abnormalities of DS include (or are the result of) a derangement of the differentiation pathway leading to maturation of fully competent T and NK cells.

In summary, the impaired NK activity of DS lymphocytes together with the T-cell defect may contribute to the high susceptibility of infection and malignancy of trisomic children.

Acknowledgements. We are grateful to Dr. Virginia Monafo for reading the manuscript. We wish to thank Attilio Ascione for his skillful technical assistance and Maria Grazia Zambon for preparation of the manuscript. This work was supported in part by CNR, Rome.

References

1. Abo T, Cooper MD, Balch CM (1982) Characterization of HNK−1+ (Leu-7) human lymphocytes I. Two distinct phenotypes of human NK cells with different cytotoxic capability. J Immunol 129:1752–1757
2. Agarwal SS, Blumberg BS, Gerstlly BJS, London WT, Sutmick J, Loeb LL (1970) DNA polymerase activity as an index of lymphocyte stimulation: studies in Down's syndrome. J Clin Invest 49: 161–169
3. Baxter R, Larkins RG, Martin FIR, Heyma P, Mylex K, Ryan L (1975) Down syndrome and thyroid function in adults. Lancet II: 794–796
4. Benda CE, Strassman GS (1965) The Thymus in mongolism. J Ment Defic Res 9:109–117
5. Bjorksten B, Back O, Gustavson KH, Hallmans G, Hagglof B, Tarnvik A (1980) Zinc and immune function in Down's syndrome. Acta Pediatr Scand 69:183–187
6. Boehmer H von, Turton K (1983) Autologous proliferating K/D restricted cytolytic T cell clones. Eur J Immunol 13:176–179
7. Boehmer H von, Kieselow P, Leiserson W, Haas W (1984) LYT-2− T cell-independent functions of LYT2+ cells stimulated with antigen or concanavalin A. J Immunol 133:59–64
8. Burgio GR, Severi F, Rossoni R, Vaccaro R (1965) Mongolism and thyroid autoimmunity. Lancet I:166–167
9. Burgio GR, Ugazio AG, Nespoli L, Marcioni AF, Bottelli AM, Pasquali F (1975) Derangements of immunoglobulin levels, phytohemagglutinin responsiveness and T and B cells markers in Down's syndrome at different ages. Eur J Immunol 5:600–603
10. Burgio GR, Lanzavecchia A, Maccario R, Vitiello A, Plebani A, Ugazio AG (1978) Immunodeficiency in Down's syndrome: T lymphocyte subset imbalance in trisomic children. Clin Exp Immunol 33:298–301
11. Burgio GR, Ugazio AG, Nespoli L, Maccario R (1983) Down syndrome: a model of immunodeficiency. Birth Defects 19:325–327
12. Cupples CG, Tan YH (1977) Effect of human interferon preparations on lymphoblastogenesis in Down's syndrome. Nature 267: 165–167
13. Duse M, Brugo MA, Martini A, Tassi C, Ferrario C, Ugazio AG (1980) Immunodeficiency in Down's syndrome: low levels of serum thymic factor in trisomic children. Thymus 2:123–131
14. Epstein LB, Epstein CJ (1980) T lymphocyte function and sensitivity to interferon in trisomy-21. Cell Immunol 51:303–318
15. Fekete G, Kulcsar G, Daun P, Nasz I, Schuler D, Dobos M (1982) Immunological and virological investigations in Down's syndrome. Eur J Pediatr 138:59–62
16. 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 22:179–191
17. Franceschi C, Licastro F, Chisicolo M, Bonetti F, Zanotti M, Fabris N, Macchegiani E, Fantini MP, Paolucci P, Masi M (1981) Deficiency of autologous mixed lymphocyte reaction and serum thymic factor levels in Down's syndrome. J Immunol 126:2161–2167
18. Funa K, Ameren G, Alm GV, Bjiorksten B (1984) Abnormal interferon production and NK cells responses to interferon in children with Down's syndrome. Clin Exp Immunol 56:493–500
19. Gaston JHS, Waer M (1985) Virus-specific MHC-restricted lymphocytes may initiate allograft rejection. Immunol Today 6:237–239
20. Gordon MC, Sinha SK, Carlson SD (1971) Antibody responses to influenza vaccine in patients with Down's syndrome. Am J Ment Defic 75:391–399

21. Griffith AW, Silvester PE (1967) Mongols and non-mongols compared in their response to active tetanus immunization. J Ment Defic Res 11:263–266
22. Gupta S, Fikzig SM, Mariano E, Quazi A (1983) Monoclonal antibody defined T cell subsets and autologous mixed lymphocyte reaction in Down's syndrome. Clin Exp Immunol 53:25–30
23. Hansson M, Beran M, Andersson B, Kiessling R (1982) Inhibition of in vitro granulopoiesis by autologous allogeneic human NK cells. J Immunol 129:126–132
24. Herbermann RB (ed) (1980) Natural killer cell mediated immunity against tumors. Academic Press, New York
25. Herbermann RB, Ortaldo JR (1981) Natural killer cells: their role in defence against disease. Science 214:381–384
26. Hersey P, Bolhuis R (1987) "Non specific" MHC-unrestricted killer cells and their receptors. Immunol Today 8:233–239
27. James K, Ritchie AWS (1984) Do natural killer cells regulate B cell activity. Immunol Today 5:193–194
28. Kaplan J (1986) NK cell lineage and target specificity: a unifying concept. Immunol Today 7:10–13
29. Karttunen R, Nurmi T, Ilonen J, Surcel HM (1984) Cell-mediated immunodeficiency in Down's syndrome: normal IL-2 production but inverted ratio T-cell subsets. Clin Exp Immunol 55:257–263
30. Krivit W, Good RA (1957) Simultaneous occurrence of mongolism and leukemia. Ann J Dis Child 94:289–293
31. Lanier LL, Cwirla S, Federspiel N, Phillips JH (1986) Human natural killer cells isolated from peripheral blood do not rearrange T cell antigen receptor beta chain genes. J Exp Med 136:209–214
32. Levin S (1987) The immune system and susceptibility to infection in Down's syndrome. In: McCoy EE, Epstein CJ (eds) Oncology and immunology of Down syndrome. Liss, New York, pp 143–162
33. Levin S, Nir E, Hogilner BM (1975) T system immune deficiency in Down's syndrome. Pediatrics 56:123–126
34. Levin S, Schlesinger M, Handzel T, Hahn T, Altman Y, Czermobilsky B, Boss J (1979) Thymic deficiency in Down's syndrome. Pediatrics 63:80–86
35. Lopez V, Ochs HD, Thuline HC, Davis SD, Wedgwood RJ (1975) Defective antibody response to bacteriophage X174 in Down's syndrome. J Pediatr 86:207–211
36. Lopez-Botet M, Silva A, Rodriguez J, Landazuri MO (1982) Generation of T cell blasts with NK-like activity in human MLC: cellular precursors, IL-2 responsiveness, and phenotype expression. J Immunol 129:1109–1115
37. Maccario R, Ugazio AG, Nespoli L, Alberini C, Montagna D, Porta F, Bonetti F, Burgio GR (1984) Lymphocyte subpopulations in Down's syndrome: high percentage of circulating HNK−1+, Leu2a+ cells. Clin Exp Immunol 57:222–226
38. Maccario R, Burgio GR (1987) T and NK lymphocyte subpopulations in the neonate. In: Burgio GR, Hanson LA, Ugazio AG (eds) Immunology of the neonate. Springer, Berlin Heidelberg New York, pp 120–129
39. Miller RW (1970) Neoplasia and Down's syndrome. Ann NY Acad Sci 171:637–644
40. Moretta A, Pantaleo G, Mingari MC, Melioli G, Moretta L, Cerottini JC (1984) Assignment of human natural killer (NK) like cells to the T cell lineage. Single allospecific T cell clones lyse specific or NK-sensitive target cells via distinct recognition structures. Eur J Immunol 14:121–125
41. Morgensen KE, Vignaux F, Gresser I (1982) Enhanced expression of cellular receptors for human interferon alpha on peripheral lymphocytes from patients with Down's syndrome. FEBS 104:285–287
42. Morling N, Jakobsen BK, Platz P, Ryder LP, Svejgaard A, Thomsen M (1982) Typing for human alloantigens with the primed lymphocyte typing technique. Adv Immunol 32:66–156
43. Nair MPN, Schwartz SA (1984) Association of decreased T-cell-mediated natural cytotoxicity and interferon production in Down's syndrome. Clin Immunol Immunopathol 33:412–424
44. Nurmi T, Huttunen K, Lassila O, Hen Honen M, Sakkinen A, Linna SL, Tijlikainen A (1982) Natural killer cell function in trisomy-21 (Down's syndrome). Clin Exp Immunol 47:735–741
45. Ortaldo JR, Herbermann RB (1984) Heterogeneity of natural killer cells. Ann Rev Immunol 2:359–394
46. Oster J, Mikkelsen M, Nielsen A (1975) Mortality and life-table in Down's syndrome. Acta Paediatr Scand 64:322–326
47. Paciucci PA, McPhail S, Zarling JM, Bach FH (1980) Lysis of syngeneic solid tumor by alloantigen stimulated mouse T and non T cells. J Immunol 124:370–375
48. Parmiani G, Sensi M, Fossati G (1985) Allostimulation induced tumor cytotoxic cells: from mouse to man. Immunol Today 6:215–218
49. Philip R, Berger AC, McNanus NH, Warner NH, Peacock MA, Epstein LB (1986) Abnormalities of the in vitro cellular and humoral responses to tetanus and influenza antigens with concomitant numerical alterations in lymphocyte subsets in Down syndrome (Trisomy-21). J Immunol 136:1661–1667
50. Phillips JH, Le AM, Lanier LL (1984) Natural killer cells activated in a human mixed lymphocyte response culture identified by expression of Leu-11 and class II histocompatibility antigens. J Exp Med 159:993–1008
51. Pistoia V, Ghio R, Nocera A, Leprini A, Perata A, Ferrarini M (1985) Large granular lymphocytes have a promoting activity on human peripheral blood erythroid burst forming units. Blood 65:464–472
52. Procopio ADG, Allavena P, Ortaldo JR (1985) Noncytotoxic functions of natural killer (NK) cells: large granular lymphocytes (LGL) produce a B cell growth factor (BCGF). J Immunol 135:3264–3271
53. Revel M, Bash D, Ruddle FH (1976) Antibodies to cell surface components codes by human chromosome 21 inhibit action of interferon. Nature 260:139–141
54. Sasaki M, Obara Y (1969) Hypersensitivity of lymphocytes in Down's syndrome shown by mixed leucocyte culture experiments. Nature 222:596–598
55. Siegel M (1984) Susceptibility of mongoloids to infections. II. Antibody response to tetanus toxoid and typhoid vaccine. Ann J Hyg 48:63–73
56. Strassmann G, Bach FH, Zarling JM (1983) Depletion of human NK cells with monoclonal antibodies allows the generation of cytotoxic T lymphocytes without NK-like cells in mixed cultures. J Immunol 130:1556–1560
57. Strausser JL, Mazumder A, Grimm EA, Lotze MT, Rosemberg SA (1981) Lysis of human solid tumors by autologous cells sensitized in vitro to alloantigens. J Immunol 127:266–271
58. Sutnik AI, London WT, Gerstly BJS, Cronlund MN, Blumberg BS (1968) Anicteric hepatitis associated with Australia antigen. JAMA 205:670–674
59. Tan YH (1975) Chromosome-21-dosage effect on inductibility of anti-viral gene (s). Nature 253:280–282
60. Tan HY (1976) Chromosome-21 and cell growth inhibitory effect of human interferon preparations. Nature 260:141–143
61. Ugazio AG, Jayakar S, Marcioni AF, Duse M, Monafo V, Pasquali G, Burgio GR (1977) Immunodeficiency in Down's syndrome: relationship between presence of human thyroglobulin antibodies and HBsAg carries status. Eur J Pediatr 126:139–146
62. Walford RL, Gessett TC, Naeim F, Tam CF, Van Laucker JL, Barnett EV, Chia D, Sparkes RS, Fahey JL, Spina C, Gatti RA, Media MA, Grossman H, Hibraur H, Motola M (1981) Immunological and biochemical studies of Down's syndrome as a model of accelerated aging. In: Segre D, Smith L (eds) Immunological aspects of aging. Dekker, New York, p 479
63. Weil J, Epstein LB, Epstein CJ (1980) Synthesis of interferon-induced polypeptides in normal and chromosome 21-aneuploid human fibroblasts: relationship to relative sensitivities in antiviral assays. J Interferon Res 1:111
64. Whittingham S, Pitt DB, Sharmay DLB, Mackay IR (1977) Stress deficiency of the T lymphocytes system exemplified by Down's syndrome. Lancet I:163–166
65. Widmer MB, Bach FH (1981) Antigen-driven helper cell-independent cloned cytolytic T lymphocytes. Nature 294:750–752
66. Yonagi Y, Caccia N, Kroanenberg M, Chin B, Roder J, Rohel D, Kiyohara T, Lanzon R, Toyonaga B, Rosenthal K, Dennert G, Acha-Orbea H, Hangartzer H, Hood L, Mark TW (1985) Gene rearrangement in cells with natural killer activity and expression of beta-chain of T-cell antigen receptor. Nature 314:631–633

Received November 24, 1987 / Accepted February 19, 1988