Autoimmune neutropenia of infancy

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Abstract

Aim: Assessment of the clinical and haematological course of autoimmune neutropenia of infancy (ANI) in a defined childhood population in the south east of Scotland.

Methods: From January 1986 to February 1991 all children presenting with persistent neutropenia were examined serologically for evidence of anti-granulocyte antibodies. The clinical course of those children found to have anti-granulocyte antibodies was then closely monitored.

Results: During the study period five children had serologically confirmed ANI, giving an annual incidence of approximately 1/100 000 in this population. All of these cases followed the classic benign course of the condition. The presenting illnesses were mild, often with superficial skin sepsis and the initial absolute neutrophil count (ANC) ranged from $0.00-0.87 \times 10^9/l$. All have remained well with no serious infections. Two children attained a normal ANC after 14 and 24 months respectively, the others currently remain neutropenic.

Conclusions: Autoimmune neutropenia of infancy is a condition which rests on a serological diagnosis. It follows a chronic benign course and all children eventually attain a normal ANC. The level of anti-granulocyte antibody in the serum often begins to wane prior to improvement in the ANC and can give an indication of when recovery will begin to occur.

Autoimmune neutropenia of infancy (ANI), first described by Lalezari in 1975,¹ is a benign condition which has become increasingly characterised as reliable techniques for detecting anti-granulocyte antibodies have become more widely available. In 1986, Lalezari *et al*² reported 121 infants with this disorder, 119 of whom were demonstrated to have anti-granulocyte antibodies. Smaller studies³⁻⁷ have confirmed both the clinical and serological features of the condition and it seems likely that the condition previously termed chronic benign neutropenia is equivalent to ANI.^{2,8,9} The exact incidence of ANI is unknown but because of its benign nature, the disorder may be more common than is suggested by the available literature.

From January 1986 to February 1991, within the Lothian region of Scotland, which has a childhood population of just over 96 000 aged

from 0-10 years, we have seen five children with serologically confirmed ANI, suggesting an annual incidence of approximately 1/1000 000 in this population. All of these cases have so far followed the classic benign course of the condition.

Methods

From January 1986 to February 1991 all children presenting outside the neonatal period with isolated neutropenia (absolute neutrophil count (ANC) $<1.5 \times 10^9/l$), were seen by OBE. A full history and examination were carried out and particular attention was paid to previous illnesses, medications administered, and family history.

Screening investigations to ascertain the cause of the neutropenia were carried out when the ANC had not returned to normal within 2-3 weeks. These included: serial full blood counts; bone marrow aspiration; serum immunoglobulin (G, A, M) and complement concentrations; lymphocyte subsets; auto-antibodies; viral serology; B12 and folate concentrations; chromosomal analysis; blood and urine amino acid screening; and anti-granulocyte antibodies. The children found to have anti-granulocyte antibodies and other features consistent with the diagnosis of ANI form the basis of this study.

Two techniques were used to detect granulocyte reactive antibodies; a microplate modification of the granulocyte immunofluorescence test (GIFT)¹⁰ and the granulocyte chemiluminescence test (GCLT).¹¹ A microplate modification of the lymphocyte immunofluorescence test (LIFT)¹² was used to detect lymphocyte reactive (anti-HLA) antibodies. Results of the immunofluorescence tests were scored as negative (0) or as graded positives ranging from W+ (weak+) to 4+. The GCLT is a functional assay which measures the response of human monocytes to opsonised granulocytes. The results of the GCLT were expressed as a ratio (the opsonic index) between the response of monocytes to granulocytes incubated with test serum and the response of monocytes to granulocytes incubated with serum from untransfused male (group AB) donors. The normal range of the GCLT is 1.0 ± 0.3 (3 SD).

The techniques were performed as previously described except for the following modifications: granulocytes and mononuclear cells used in both techniques were isolated from EDTA anticoagulated blood from normal donors using a double density gradient technique. Red cells contaminating the granulocyte

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Table 1 Cases of autoimmune neutropaenia of infancy in south east Scotland (1986–1991)

Cases	Age at onset (months)	Sex	Presenting illness	ANC	TWC	Initial granulocyte serology	Lymphocyte subsets T4:T8	Serum IgG IgM IgA	Bone marrow		Increased infections during ANI	Time to normal ANC (months)
				TWC	diff (%)				M:E	Maturation arrest		
1	13	F	Gastroenteritis	0.0 6.2	N-0 L-86	OI-10.0 Antig IgG 3+ no specific target Ag identified	9.83:1	N	6.5:1	NO	NO	24
2	8	M	Fever and skin sepsis	0.19 4.8	N-4 L-68 M-26 myelo-1 meta-1	OI-12.8 Antig IgG 3+ antig IgM W+ no specific target Ag identified	4.6:1	N	8.5:1	NO	NO	Not yet recovered after 19 months
3	8	M	Skin sepsis, lymphadenitis, upper respiratory tract infection	0.87 8.7	N-10 L-62 M-27 E-1	OI-8.6 Antig IgG 3+ antig IgM 0 target Ag NA1	4.3:1	N	9.4:1	NO	NO	Not yet recovered after 11 months
4	11	F	Skin sepsis	0.13 4.4	N-3 L-80 M-12 E-4 B1	OI-15.9 Antig IgG 2+ Antig IgM 0 target Ag NA1	2.7:1	N	2.5:1	NO	NO	Not yet recovered after 7 months
5	7	F	Fever, vomiting oral/anal candidiasis	0.0 10.9	N-0 L-91 M-9	OI-1.2 Antig IgG 1+ Antig IgM NEG target Ag NA1	2.7:1	N	1.33:1	NO	NO	14

ANC = absolute neutrophil count $\times 10^9/l$; TWC = total white count $\times 10^9/l$; TWC percentage differential: N = neutrophils; L = lymphocytes; M = monocytes; Myelo = myelocytes; Meta = metamyelocytes; E = eosinophils; B = basophils. OI = opsonic index; Antig = antigranulocyte; Ag = antigen; N = normal.

fraction were lysed using a hypo-osmolar solution of ammonium oxalate. Granulocytes were inactivated by incubation at 52°C for 2 minutes prior to incubation with test serum and were not treated with paraformaldehyde.

Target granulocytes and lymphocytes were obtained from normal donors typed for the neutrophil specific antigens NA1, NA2, and NB1, the tissue antigen 5b and HLA-A and B antigens. Granulocyte immunofluorescence was used to determine NA1, NA2, NB1, and 5b phenotype and lymphocytotoxicity was used to determine HLA-A and B phenotypes.

Results

During the study period, within the Lothian region of Scotland which has a childhood population of just over 96 000 aged from 0–10 years, five children presented with serologically confirmed ANI (table 1). Three were female and the median age at diagnosis was 8 months (range 7–13 months). They all presented with minor illnesses, and in three there was skin sepsis. All had absolute neutropenia with ANC ranging from 0.00–0.87 $\times 10^9/l$, case 4 also had iron deficiency anaemia. Bone marrow examination of these patients confirmed an increased M:E ratio with “left shift” of the myeloid series but no specific arrest patterns. In cases 1–3 there appeared to be an initial elevated T4:T8 lymphocyte subset ratio which normalised with time. Serum immunoglobulin concentrations were normal.

The anti-granulocyte antibodies detected were of the IgG class but weakly reactive IgM antibodies were also detected in one patient. In three patients the anti-granulocyte antibodies were specific for the NA1 antigen. Anti-HLA antibodies were not detected.

In cases 1 and 5 the neutropenia has resolved after 14 and 24 months respectively; in the other three cases neutropenia persists at

present. None of these children has suffered any severe infections or any great increase in minor infections during the course of the neutropenia. Case 1 has been closely followed through to complete recovery and the clinical course of this patient demonstrates the typical features of this disorder (figure):

(1) The total white blood count always remained within normal limits.

(2) The ANC remained below $0.5 \times 10^9/l$ for the first 12 months.

(3) The level of anti-granulocyte antibody activity slowly decreased during the course of the disease.

(4) Temporary increases in peripheral neutrophil counts were observed with the development of *Campylobacter* enteritis and an urticarial illness.

(5) The incidence of infections (five upper respiratory tract infections, two fevers, one episode of mouth ulcers and one episode of diarrhoea, in addition to the two illnesses described above) during the 24 month period of neutropenia could not be considered excessive for a child of this age.

Discussion

The five patients described in this report have clinical and laboratory features (table 1) entirely consistent with the previous reports of ANI.^{2–7} These five cases, from a defined geographical area, suggest that the annual incidence of ANI is in the order of 1/100 000 of the childhood population, although, because of the relatively benign course of the disorder this figure is likely to be an underestimate.

The differential diagnosis of neutropenia in infancy was recently reviewed and an algorithm for assessment of such infants described.¹³ The many causes of infant neutropenia can be divided into congenital and acquired (table 2). The congenital syndromes may be associated with phenotypic abnormalities, immuno-

Typical course of autoimmune neutropenia of infancy (case 1).

deficiency, or be pure neutrophil abnormalities. This group of patients tends to present with serious infections in the very early months of life, with the exception of cyclical neutropenia which may present later with less serious infections, often superficial, and often with a positive family history.¹⁴ Clues to the acquired causes may be elicited from the history and consequent appropriate investigations.

The diagnosis of ANI depends on history and examination and upon positive serological results, although these will not be available at the time of presentation. Autoimmune neutropenia has been reported in children and adults infected with the human immunodeficiency virus, so evidence for this infection should also be sought.^{15 16}

The bone marrow findings in ANI are not diagnostic and any process causing increased synthesis (for example, infection) or increased destruction of neutrophils (for example, autoimmune neutropenia) will give a similar picture. Marrow examination excludes neutropenia caused by proliferative disorders or aplasia.

Clinical features of autoimmune neutropenia of infancy

All patients have severe neutropenia at presentation; the ANC is usually less than $0.5 \times 10^9/l$ but the total white blood count is always within

normal limits. Monocytosis or eosinophilia may occur but do not seem to affect the rate of infection. Anaemia has been reported either due to coincidental iron deficiency or as a result of recurrent infections. In a study of 121 children,² the median age of diagnosis was 8 months (range 3–30) and the female to male ratio was 6:4. All children had ANC of less than $1.5 \times 10^9/l$ but in many cases neutrophils were undetectable. None of the children had any other serious illnesses; there was no evidence of other autoimmune diseases; no previous blood transfusions; and no drug ingestion that might have given rise to antibody formation. Most of the children presented with minor infections such as otitis media, gingivitis, respiratory tract infections, gastroenteritis, or skin sepsis. Diagnosis was often made only after the child had suffered several infections or an incidental blood count revealed neutropenia. More seriously affected children have presented with pneumonia, central sepsis, or abscesses (one study showed an increase in vulval abscesses in girls, caused particularly by *Pseudomonas aeruginosa*.⁹). No cases of ANI so far described have died as a consequence of their disease.

Longitudinal studies^{3–6} of infants with ANI have demonstrated the clinical course of the disorder. The median duration of disease is around 30 months (range 6–60 months) but 95% of children recover by 4 years of age.² Thus far no children who have recovered from this condition have been described with any secondary recurrence of neutropenia or other autoimmune problems. Neutropenia in ANI can be profound but increased neutrophil production can occur during infections, especially as antibody levels decrease. Some children show an increased level of minor infections during the course of the disease while others remain well. Generally, no treatment, apart from efficient personal hygiene and occasional antibiotics (usually oral) for minor infections, is required. Children who are subject to more serious recurrent infections may be treated with intravenous human immunoglobulin (IVIG) to induce temporary increases in the ANC. IVIG treatment has been used effectively for acute infections, surgical interventions, and for longer term protection until there is spontaneous improvement in the ANC.^{17–19}

Bone marrow aspiration has been performed in almost all children with ANI so far reported, primarily to exclude other causes of neutropenia. In ANI, the bone marrow tends to show an active picture with no abnormalities of cell morphology. There is an increased myeloid to erythroid ratio (M:E ratio) and some patients have been reported to show arrest of maturation of the myeloid series at various stages,⁵ but this does not correlate with any difference in clinical severity.⁴ Adrenaline and hydrocortisone stimulation of the bone marrow can cause increased release of neutrophils into the circulation but the response is usually poor or negligible.²⁵ These findings are not diagnostic for ANI, which can only be confirmed by the presence of anti-granulocyte antibodies.

Lalezari *et al*¹² found that serum immunoglobulin levels were normal in patients with

Table 2 Causes of neutropenia in infancy

Congenital	Acquired
Neutrophil abnormality only	Infection related
Kostmann's syndrome (severe congenital neutropenia)	Viral
Cyclical neutropenia	Bacterial
Myelokathexis	Rickettsial
Lazy leucocyte syndrome	Fungal
	Parasitic
Associated with lymphocyte abnormalities	Immune mediated
Reticular dysgenesis	Iso-immune
Agammaglobulinaemia	(materno-fetal, multi-transfusion)
X-linked agammaglobulinaemia	Auto-immune
Dysgammaglobulinaemia	(ANI, SLE, HIV)
Associated with other conditions	Marrow infiltration
Cartilage hair hypoplasia	Leukaemia
Schwachmann syndrome	Lymphoma
Fanconi's syndrome	Neuroblastoma
Chediak-Higashi syndrome	
Amino acidopathies	Marrow aplasia
	Vitamin deficiency
	Vitamin B12
	Folate
	Copper
	Drugs and toxins

ANI but, in a study of 14 patients, Bussel *et al*⁴ found that one had elevated total immunoglobulin levels and four had elevated IgM levels. In four patients total IgG levels were normal but decreased levels of IgG2 and IgG3 were found. The T4:T8 lymphocyte ratio was within normal limits in 10/121 cases tested.² Assessment of neutrophil function has not shown any conspicuous abnormality, although only very small numbers of patients have been examined.⁴ Serum granulocyte colony-stimulating factor levels are elevated in children with ANI and fall as the ANC improves, implying an autonomous feed back response in this condition.²⁰

Granulocyte serology

Anti-granulocyte antibodies detected in cases of ANI are usually of the IgG class, but may occur together with IgM antibodies.² These antibodies often have specificity for the NA1 antigen, although other specificities have been reported.²¹ In Lalezari's study,² antibodies specific for NA1 (or which had NA1-related activity) were identified in 17/121 patients, while in another study⁷ anti-NA1 antibodies were identified in 17/36 patients.

Many different techniques have been described for the detection of granulocyte reactive antibodies,⁷ but in the first international granulocyte serology workshop,²² the granulocyte agglutination test and the granulocyte immunofluorescence test were the most widely used and reliable techniques. The most successful laboratories in this workshop used more than one technique for the investigation of antisera and it is clear that no single technique can detect all types of granulocyte reactive antibodies.

The initiating process in the development of anti-granulocyte antibodies is still unknown. It may be speculated that ANI results from an unusual response to common infection, as has been postulated in the pathogenesis of acute idiopathic thrombocytopenic purpura of childhood.²³ The high incidence of antibodies specific for NA1 in this disorder may also provide a key to the understanding of this condition. Studies into the nature of the neutrophil antigens and the receptors on the neutrophil surface^{24,25} have shown that the neutrophil specific antigens NA1 and NA2 are closely bound to the neutrophil Fc γ receptor 111 (CD16) for immunoglobulin G (FcR 111). FcR 111 is a low affinity receptor for IgG and binds IgG1 and IgG3 dimers only. Expression of this receptor by neutrophils can be increased by cytokines released during the inflammatory process. The neutrophil can also secrete FcR 111 in soluble form when stimulated. This receptor may not only have a role in the cellular immune system inducing phagocytosis and antibody dependent cellular cytotoxicity but, when released, may act as a ligand and modulate IgG production by lymphocytes.²⁶ The possible interrelationship(s) between the NA1/NA2 antigens, the FcR III receptor, and the anti-neutrophil antibodies in ANI remains to be elucidated.

Conclusion

ANI is a benign condition which follows a relatively chronic course with all children

recovering a normal ANC by at least 5–6 years of age. Our study suggests that the disease has an annual incidence in the order of 1/100 000 of the childhood population. The diagnosis of ANI depends on serological confirmation of anti-granulocyte antibodies. Bone marrow aspiration is mandatory to exclude other more serious causes of neutropenia.

Children symptomatically more severely affected can be treated with IVIG to improve their neutrophil count; this can be given for an acute crisis or regularly to maintain a higher ANC. The level of anti-granulocyte antibody in the serum often begins to wane prior to improvement in the ANC and this can give an indication of when recovery will begin to occur. There are no known sequelae of this condition.

- 1 Lalezari P, Jiang AF, Yegen L, Santorineou M. Chronic autoimmune neutropenia due to anti-NA2 antibody. *N Engl J Med* 1975;293:744–7.
- 2 Lalezari P, Kharshidi M, Petrosova M. Autoimmune neutropenia of infancy. *J Pediatr* 1986;109:764–9.
- 3 Conway LT, Clay ME, Kline WE, Ramsay NKC, Krivit W, McCullough J. Natural history of primary autoimmune neutropenia in infancy. *Pediatrics* 1987;79:728–33.
- 4 Bussel JB, Abboud MR. Autoimmune neutropenia of childhood. *CRC Crit Rev Oncol Hematol* 1987;7:37–51.
- 5 Onisawa S, Ichimura T, Saito K, Sekine I. Five cases of autoimmune neutropenia in infancy or early childhood. *Acta Paediatr Jpn [Overseas Ed]* 1989;3:587–94.
- 6 Ducos R, Madyastha PR, Warrior RP, Glassman AB, Shirley LR. Neutrophil agglutinins in idiopathic chronic neutropenia of early childhood. *Am J Dis Child* 1986;140:65–8.
- 7 McCullough J, Clay M, Press C, Kline W. In: *Granulocyte serology: a clinical and laboratory guide*. Chicago: American Society of Clinical Pathologists Press, 1988.
- 8 Madyastha PR, Fudenberg HH, Glassman AB, Madyastha KR, Smith CL. Autoimmune neutropenia in early infancy: a review. *Ann Clin Lab Sci* 1982;12:356–67.
- 9 Jonsson OG, Buchanan GR. Chronic neutropenia during childhood. A 13-year experience in a single institution. *Am J Dis Child* 1991;145:232–5.
- 10 Verheugt FWA, von dem Borne AEGKr, van Noord-Bokhorst JC, Engelfriet CP. Autoimmune neutropenia: The detection of granulocyte autoantibodies with immunofluorescence test. *Br J Haematol* 1978;39:339–50.
- 11 Hadley A, Holburn AM. The detection of anti-granulocyte antibodies by chemiluminescence. *Clin Lab Haematol* 1984;6:351–61.
- 12 Decary F, Vermeulen A, Engelfriet CP. A look at HL-A antisera in the indirect immunofluorescence technique (IIFT). In: Amos DB, Van Rood JJ, eds. *Histocompatibility testing*. Copenhagen, Munksgaard: 1975:380–90.
- 13 Roskos RR, Boxer LA. Clinical disorders of neutropenia. *Pediatr Rev* 1991;12:208–12.
- 14 Wright DG, Dale DC, Fauci AS, Wolff SM. Human cyclic neutropenia: clinical review and long term follow up of patients. *Medicine* 1981;60:1–13.
- 15 McCance-Katz EF, Hoecker JL, Vitale NB. Severe neutropenia associated with anti-neutrophil antibody in a patient with acquired immunodeficiency syndrome related complex. *Ped Infect Dis J* 1987;6:417–8.
- 16 Lobut JB, Reinert P, Kohout G, Lemerle S, Bernaudin F, Cohen R. Autoimmune neutropenia disclosing AIDS in a child. *Ann Med Intern (Paris)* 1987;138:416–8.
- 17 Bussel J, Lalezari P, Fikrig S. Intravenous treatment with gamma globulin of autoimmune neutropenia of infancy. *J Pediatr* 1988;112:298–301.
- 18 Hilgartner MW, Bussel J. Use of intravenous gamma-globulin for the treatment of autoimmune neutropenia of childhood and autoimmune hemolytic anaemia. *Am J Med* 1987;83 (Suppl 4A):25–9.
- 19 Kurtzberg J, Friedman HS, Chaffee S, *et al*. Efficacy of intravenous gamma globulin in autoimmune mediated pediatric blood dyscrasias. *Am J Med* 1987;83 (Suppl 4A):4–9.
- 20 Mizuno Y, Hara T, Nagata M, *et al*. Serum granulocyte colony-stimulating factor levels in chronic neutropenia of infancy. *Pediatr Hem Oncol* 1990;7:377–81.
- 21 McCullough J. Autoimmune granulocytopenia. In: Engelfriet CP, van Loghem JJ, von dem Borne AEG Kr, eds. *Research monographs in immunology. Vol 5. Immunohaematology*. Amsterdam: Elsevier, 1984:257–4.
- 22 Lucas GF, Carrington PA. Results of the first granulocyte serology workshop. *Vox Sang* 1990;59:251–6.
- 23 Lusher JM, Iyer R. Idiopathic thrombocytopenic purpura in children. *Semin Thromb Hemost* 1977;33:175–99.
- 24 Rothko K, Kickler TS, Clay ME, Johnson RJ, Stroncek DF. Immunoblotting characterisation of neutrophil antigenic targets in autoimmune neutropenia. *Blood* 1989;74:1698–703.
- 25 Huizinga TWJ, Kleijer M, Tetteroo PAT, Roos D, von dem Borne AEG Kr. Biallelic neutrophil NA-antigen is associated with a polymorphism on the phosphoinositide-linked Fc gamma Receptor 111 (CD16). *Blood* 1990;75:213–7.
- 26 Huizinga TWJ, Roos D, von dem Borne AEG Kr. Neutrophil Fc gamma receptors: a two way bridge in the immune system. *Blood* 1990;75:1211–4.