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(Accepted 27 April 1992)

Detection of IgA and IgM antibodies to HIV-1 in neonates by radioimmune western blotting

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Abstract

Objective—To detect infection with HIV-1 by IgA and IgM response at birth in children born to HIV-1 seropositive mothers.

Design—Western blotting and radioimmune western blotting on stored sera from infected and uninfected babies born to HIV-1 seropositive mothers. Sera were pretreated to remove IgG.

Setting—Parma and Bologna, Italy.

Subjects—12 infected and five uninfected babies born to HIV-1 seropositive mothers and three babies born to seronegative mothers.

Main outcome measures—Effectiveness of western blotting and radioimmune western blotting in detecting antibodies to HIV-1 gene products.

Results—With conventional western blotting we found IgA class antibodies to HIV-1 proteins in serum from three out of 12 infected children; in two of these three the serum was collected at age 3 months (positive controls). Radioimmune western blotting detected both IgA and IgM antibodies in serum from all infected children tested, whereas all serum from uninfected children born to seropositive and seronegative mothers showed no such antibodies.

Conclusion—Although the technique should be tested on more patients, radioimmune western blotting seems to be a valuable tool for serological diagnosis of congenital HIV-1 infection at birth in neonates born to seropositive mothers.

Introduction

By the end of this century AIDS may be the main cause of death among children.¹ Around 15-30% of babies born to seropositive mothers are infected with HIV, and among these 83% will show clinical or laboratory signs by the age of 6 months.^{2,3}

Early diagnosis of HIV-1 infection is an essential requisite to intervention with antiviral or other treatment because the clinical symptoms in infected children often appear late in the course of infection. Many methods have thus been used to define an infected state. Viral culture has proved sensitive, but it requires one to four weeks to become positive, necessitates special biosafety precautions, is costly and labour intensive,^{4,5} and not all infected children give positive cultures at birth.^{6,7}

The p24 antigen assay is of limited use because of the presence of immune complexes which may mask the HIV-1 antigen.^{8,9} The "in vitro antibodies production assay"¹⁰⁻¹² and the related Elispot technique¹³ detect lymphocytes producing HIV-1 antibodies, but this method is only effective after the first month of life.

The polymerase chain reaction is the only technique

that permits a rapid and low cost diagnosis within the first two months of life and requires only 1-3 ml of blood,^{8,9,14,15} but the results are not always reliable because of false positive results.¹⁶

Serological tests form the basis for the diagnosis of HIV infection in adults, but in children born to seropositive mothers they are hampered by the presence of passively transmitted IgG antibodies, which may be detectable up to 18 months after birth.^{2,8,17}

Since IgA and IgM antibodies do not cross the placenta they are a reliable sign of infection but their small amount as well as the transient production of IgM antibodies and the presence of maternal IgG may render enzyme linked immunosorbent assays (ELISA) and western blot assays unreliable. Removal of IgG with recombinant protein G allows detection of specific IgA in most infected children aged at least 12 months; in younger children, however, the results are less reliable and the positive results of IgM detection are reduced by up to half with IgA antibodies,^{18,19} probably because of the low sensitivity of the method used.

We thus developed a more sensitive method to detect antibodies to HIV-1 proteins; it consists of a modified western blot procedure (radioimmune western blotting) in which biotinylated antibodies to HIV-1 antibodies are detected by means of isotope (sulphur-35 or iodine-125) labelled streptavidin.²⁰ We used both western blotting and radioimmune western blotting on stored serum from infected (G Furlini, *et al*, seventh international conference on AIDS, Florence, 1991; abstract No WA 1340) and uninfected children born to seropositive mothers as well as on serum from seronegative children born to mothers without any history of HIV-1 infection to search for HIV-1 specific IgA and IgM antibodies.

Subjects and methods

SUBJECTS

This unblinded survey was performed on 70 children born to seropositive mothers in Bologna. Infection had been verified in only 12 (17%); the remaining 58 (83%) were uninfected as determined by viral culture or p24 antigen assay, or both. For 10 out of the 12 infected children the serum was collected at birth. In the remaining two serum was collected aged 3 months to guarantee the presence of IgA or IgM antibodies to HIV-1 protein, or both, and represented our positive controls for infected children in radioimmune western blotting. Five of the 58 uninfected children were randomly selected as negative controls for uninfected children born to seropositive mothers. Serum from three children born to seronegative mothers were tested as further controls. All serum

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BMJ 1992;304:1539-42

from uninfected children was taken within the first month of life.

IgG REMOVAL WITH RECOMBINANT PROTEIN G

Protein G Sepharose 4 Fast Flow (Pharmacia, Italy) was used to remove IgG antibodies from the serum. Briefly, 300 µl of serum was added to 900 µl of protein G diluted in phosphate buffer saline, sufficient to bind 9 mg of IgG. This amount of protein G is able to bind double the concentration of IgG antibodies normally present in children's serum at birth.

Serum from the positive control children (collected aged 3 months) was treated twice with protein G because of its relatively high titre of IgG antibodies, although we used the same final dilution (1 in 32) as for the other sera. After overnight incubation at room temperature with gentle constant mixing the sample was centrifuged for three minutes in a microfuge at 1000 g. This resulted in about 600-700 µl of supernatant.

WESTERN BLOTTING AND RADIOIMMUNE WESTERN BLOTTING

For western blotting and radioimmune western blotting we used strips blotted with viral antigens, wash buffer, milk buffer, biotinylated antibodies to IgG, and 4-chloro-1-naphthol from DuPont de Nemours (Brussels, Belgium). Biotinylated antibodies to IgA and IgM (1 in 100 dilution in milk buffer), streptavidin peroxidase (1 in 500 in milk buffer), and ¹²⁵I streptavidin (2 µCi/strip in milk buffer) were obtained from Amersham (Amersham, Buckinghamshire). Streptavidin peroxidase gave the same results, in terms of specificity and sensitivity, as obtained with avidin peroxidase (data not shown).

After removal of IgG the serum was diluted in milk buffer to give a final dilution of 1 in 32 (for both IgA and IgM assays) and incubated with the strips overnight in a shaker at room temperature. After this incubation period the strips were washed 10 times for five minutes before the first conjugate (biotinylated antibodies to IgA or IgM) was added. After a one hour incubation period the strips were washed as above and

then incubated with the second conjugate (streptavidin peroxidase for western blot or ¹²⁵I streptavidin for radioimmune western blot for one hour with gentle shaking.

The strips conjugated with streptavidin peroxidase were washed as above and received in conventional western blot 4-chloro-1-naphthol as peroxidase substrate for 10 minutes; peroxidase staining was stopped by three washings of five minutes with distilled water. The strips conjugated with ¹²⁵I streptavidin were air dried after extensive washing with distilled water and examined by autoradiography. The autoradiographic sheet was developed and fixed after a five day exposure.

Results

Figures 1 and 2 show the autoradiographs of IgA and IgM antibodies found in infected children by radioimmune western blotting and the corresponding data obtained with conventional western blotting. Table I summarises all data obtained from serum of infected (1-12) and uninfected (13-20) children.

Using conventional western blotting, we found IgA class antibodies in three (25%) out of 12 sera whereas IgM antibodies were detected in only one and were directed against a single HIV-1 protein (p24). With radioimmune western blotting serum from all infected children showed both IgA and IgM antibodies to one or more HIV-1 protein whereas serum from all uninfected children had no such antibodies.

Only one of the infected children had IgA antibodies to just a single HIV-1 protein (gp160 in child 8); the 11 others had antibodies to two or more specific HIV-1 proteins. IgM antibodies to a single HIV-1 protein were detected in only two cases and antibodies to at least two or more specific HIV-1 proteins in 10 cases. The HIV-1 proteins most commonly detected by both radioimmune western blotting for IgA and IgM were Env(gp160, gp120) and Gag (p24, p17) gene products. These data are in agreement with those of studies on sera obtained close to the seroconversion time.²¹

In addition, radioimmune western blotting detected an almost complete antibody pattern; 91% (11) of

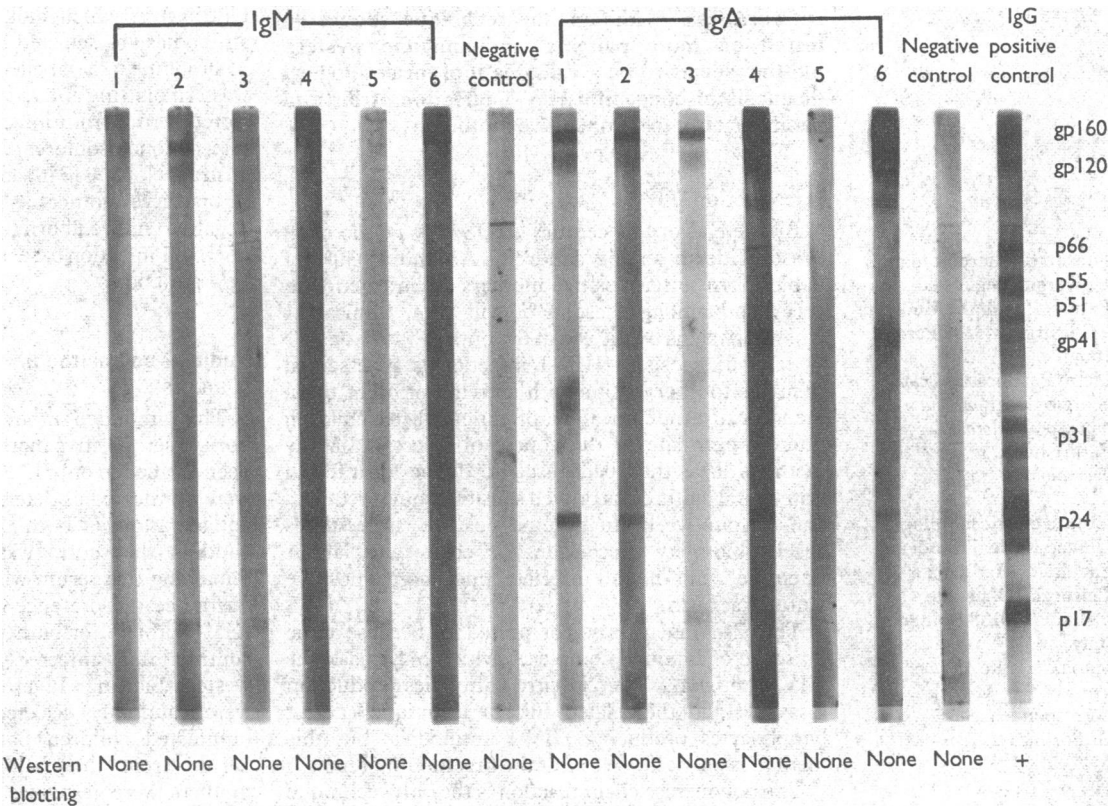


FIG 1—Radioimmune western blot showing IgM and IgA antibodies to HIV-1 proteins in infected children born to seropositive mothers and antibodies detected by western blotting. Strips 1-6 correspond to patients 1-6 in table. Strip 4 is from serum of infected child aged 3 months

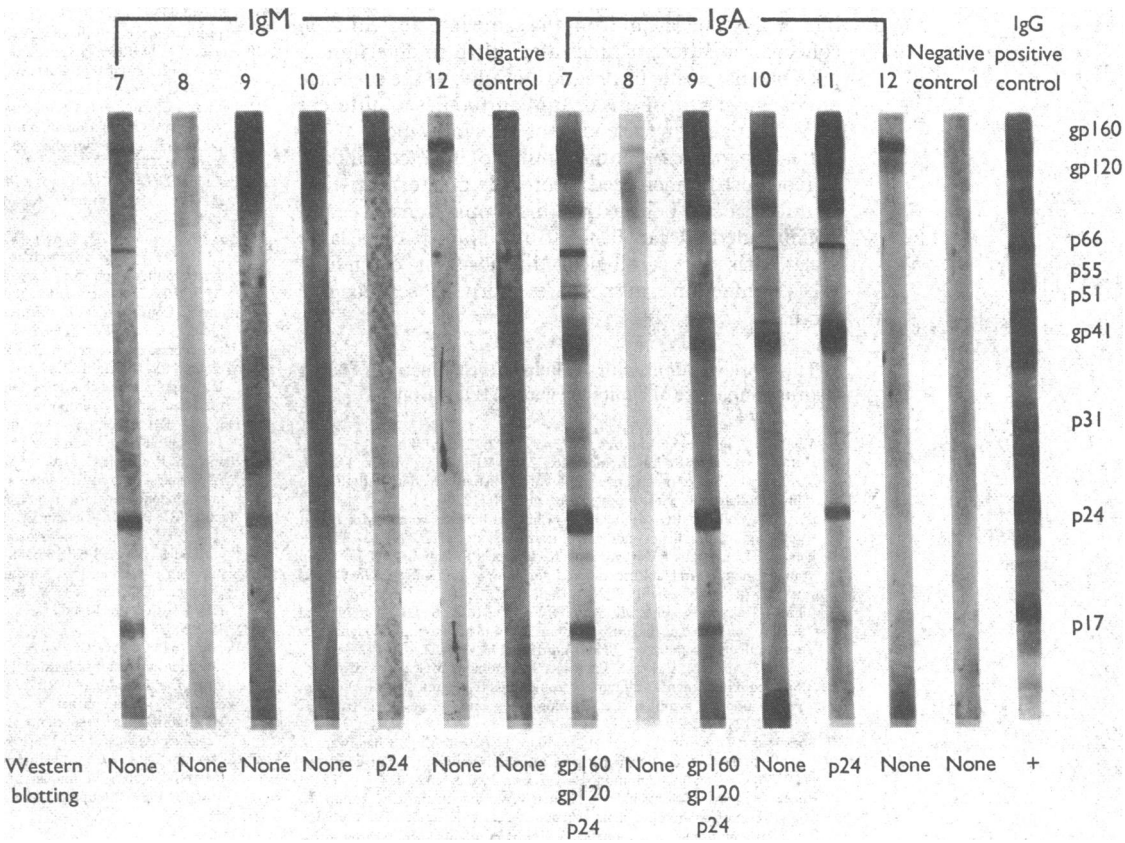


FIG 2—Radioimmune western blot showing IgM and IgA antibodies to HIV-1 proteins in infected children born to seropositive mothers and antibodies detected by western blotting. Strips 7-12 correspond to patients 7-12 in table. Strip 7 is from serum of infected child aged 3 months

IgA and IgM antibodies to HIV-1 proteins in children by western blotting and radioimmune western blotting. Patients 1-12 were infected and sera were collected at birth except for patients 4 and 7 (aged 3 months). Patients 13-17 were uninfected but born to seropositive mothers and patients 18-20 were born to seronegative mothers

Patient No	IgA antibodies to HIV-1 proteins		IgM antibodies to HIV-1 proteins	
	Western blotting	Radioimmune western blotting	Western blotting	Radioimmune western blotting
1		gp160, gp120, p24		gp160, p24*, p17*
2		gp160, gp120, p24, p17		gp160, gp120, p24, p17
3		gp160, gp120, p17		gp160, p17
4		gp160, gp120, p66, gp41, p24, p17		gp160, gp120, p24, p17
5		gp160*, p24*, p17*		p24, p17
6		gp160, gp120, gp41, p24		gp160, gp120, p17
7	gp160, gp120, p24	gp160, gp120, p66, p55, p51, gp41, p31, p24, p17		gp160, gp120, p66, p24, p17
8		gp160		gp160
9	gp160, gp120, p24	gp160, gp120, p66, p55, gp41, p31, p24, p17		gp160, p24
10		gp160, gp120, p66, p51, gp41, p31*, p24*		gp160
11	p24*	gp160, gp120, p66, gp41, p24	p24*	gp160, gp120, p24
12		gp160, gp120		gp160, gp120
13				
14				
15				
16				
17				
18				
19				
20				

*Weak intensity.

infected serum samples in the IgA assay and 67% (eight) in the IgM assay met the positive criterion established for IgG western blotting by the Centers for Disease Control.²²

Discussion

We have described an application of radioimmune western blotting for diagnosis of congenital HIV-1 infections at birth and compared it with conventional western blot analysis.

Our conventional western blotting results agreed with other findings by the same technique¹⁸; Weiblen *et al* established positivity even in the presence of only a single band and found, in infected babies aged less than 3 months, positive results for IgA in only two out of 64 and for IgM in only one out of 64 babies.¹⁸ Schubach *et al* showed a more consistent finding of IgA and IgM

antibodies to HIV-1 proteins in cord serum from children born to seropositive mothers, but the infection in the children was not assessed by an alternative method. Inexplicably, few or no IgA and IgM antibodies to Env gene products were detected.

In contrast radioimmune western blotting detected IgA and IgM antibodies in all serum collected at birth from infected children; the IgA class antibodies were directed against more proteins than IgM antibodies, probably because of a temporary loss of IgM response. Almost all HIV-1 proteins had IgA or IgM antibodies directed against them, notably Env and Gag gene products. Moreover all IgM antibodies identified were also present in the IgA assay of the same serum sample, showing complete matching of the results.

At present, however, there seems to be no correlation between the recovery of IgA or IgM antibodies to particular HIV-1 proteins and the onset of clinical

signs. We are thus carrying out radioimmune western blot analysis of serum from the same infected children collected at different times after birth to determine a possible diagnostic or prognostic value to the presence or change in titre of IgA or IgM antibodies to different HIV-1 antigens in the course of the syndrome.

Bearing in mind that the number of infected children tested must be increased to provide greater statistical significance, we believe that this simple, sensitive, and specific method can be fruitfully applied on a large scale basis for serological diagnosis of congenital HIV-1 infections in neonates born to seropositive mothers.

This work was done with the help of the Ministry of Health (Istituto Superiore di Sanità Peogetto AIDS), Rome.

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(Accepted 2 April 1992)

Endoscopy facilities in general practice

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The range of investigative aids used by general practitioners has expanded considerably in recent years, though little is known of their availability in practices or the extent to which they are used.^{1,2} I surveyed the availability and use of two such aids, the proctoscope and the sigmoidoscope, in general practices in the Northern region of England. The survey also asked about the potential effects of the new general practitioner contract and fundholding proposals on the use of these instruments.

Subjects, method, and results

A questionnaire was mailed to all 403 practices in the Northern region of England in the summer of 1989 with a reminder six weeks later. A total of 326 practices (81%) replied, representing 1144 family doctors.

In all, 234 (72%) practices had a proctoscope,

though the proportion was lowest for three person practices and highest for those with six or more partners. The proctoscope was used by all the partners in 182 (78%) practices and by no partners in 11 (5%) practices.

Only 13 (4%) of the responding practices offered rigid sigmoidoscopy as a surgery procedure. The proportion for practices with >9000 patients was higher (11%). In three practices more than one partner performed this procedure. No practice offered flexible sigmoidoscopy as a surgery procedure.

Open access to hospital gastrointestinal endoscopy services did not influence the availability of either proctoscopy or rigid sigmoidoscopy in the practices surveyed. At least one partner in 31 (10%) practices had relevant training in these procedures. Most common was surgical experience to registrar level or the FRCS, followed by practical experience of endoscopy as a clinical assistant.

Opinions in favour of the use of these diagnostic aids in family practice were expressed by 134 (41%) responding practices, the most common being general approval or that their use should be encouraged (66). Others were in favour provided appropriate training was available (32) or if the skills already existed in the practice (36). Opinions against their use in primary care were expressed by 144 (44%) practices, the most common being that they were not appropriate procedures for primary care (35), or were a specialist procedure (28). Others expressed concern that standards would be difficult to maintain (35) or that time was not available for these procedures (20). No opinion was expressed by 48 (15%) practices.

Forty three practices expressed views on the likely effect of the new general practitioner contract and proposals for fundholding. Positive responses came mainly from larger practices whose interest lay in the potential for saving money as fundholders or for increased income through payments for minor surgical

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BMJ 1992;304:1542-3

Availability of proctoscopes and sigmoidoscopes in 326 general practices in Northern region

No of partners in practice	No of practices	No (%) owning proctoscope	No (%) owning sigmoidoscope	No (%) of practices in which one partner had further training
1	52	37 (71)	1 (<1)	3 (<1)
2	61	44 (72)	4 (1)	4 (1)
3	72	44 (61)	1 (<1)	6 (2)
4	52	37 (71)	2 (<1)	5 (2)
5	37	28 (76)	1 (<1)	4 (1)
≥6	52	44 (85)	8 (15)	9 (3)
Total	326	234 (72)	13 (4)	31 (10)