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ANTI-HTLV-I p19 SUPPRESSES HTLV-I AG EXPRESSION IN PERIPHERAL BLOOD LYMPHOCYTES FROM HTLV-I CARRIERS

YOSHIYA ANDO
Department of Obstetrics and Gynecology, Wakayama Medical University, Kihoku Hospital

YOSHINARI MATSUMOTO
Department of Obstetrics and Gynecology, Osaka City University, Medical School

KAZUHIRO KAKIMOTO
Division of International Co-Operation, International Medical Center of Japan

CHIAKI MARUYA
Department of Obstetrics and Gynecology, Ohyodo Municipal Hospital

MASAHIRO ENOMOTO
Department of Obstetrics and Gynecology, Saisei-kai Chuwa Hospital

TETSUNORI MATSUDA
Department of Obstetrics and Gynecology, Shingu Municipal Medical Center

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Abstract : Anti-HTLV-I antibodies possess an inhibitory action for HTLV-I infection. In cases where cells persistently expressing HTLV-I antigen are the source of infection in an in vitro system, antibodies against HTLV-I env antigens (such as anti-HTLV-I $gp46^{175-199}$ antibody, etc.) have an inhibitory action for HTLV-I cell to cell infection. Our examination of this inhibitory action for HTLV-I infection in peripheral blood lymphocytes from HTLV-I carriers, who did not express antigens, revealed that anti-HTLV-I $p19^{100-130}$ antibody inhibit infection by suppressing the expression of HTLV-I antigens.

Key words : HTLV-I , antigen, anti-p19, anti-gp46

INTRODUCTION

Through an experimental system with XC cell (a rat sarcoma cell line) assay in vitro[1-7] and with rabbits in-vivo[8,9], anti-HTLV-I antibodies have been reported to have an inhibitory action for HTLV-I infection. It has also been reported that preventive effects in-vitro exist in the antibody fraction against HTLV-I gp46[10]. These reports suggest that the expression of the HTLV-I envelope antigen has a significant role in the intercellular infection with HTLV-I. If the expression of this HTLV-I envelope antigen can be suppressed, there is a

possibility of preventing the intercellular infection with HTLV-I. We examined what kind of antibody fraction in the anti-HTLV-I antibody controlled this antigen expression.

METHODS

We collected and pooled, with their consent, the sera samples of pregnant women who were found to be anti-HTLV-I sero-positive[11] in early-stage pregnancy screening tests. We separated using a method by Lammler et al.[12] the antibodies against HTLV-I $p19^{100-130}$ (amino acid sequence PPPPSSPTHDPPDSDPQIPPPYVEPTAPQVL)[13,14] and against HTLV-I $gp46^{175-199}$ (amino acid sequence FLNTEPSQLPPTAPPLLPHSNLDHI)[15] from the pooled anti-HTLV-I positive sera. The purity levels of these antibodies were examined using a western blot assay kit (Fujirebio Inc., Tokyo, Japan).

We separated peripheral blood lymphocytes (PBLs) from the bloods of three consenting HTLV-I sero-positive pregnant women using the Ficoll-Conray gradient centrifugation method. Then, PBLs were cultured in different types of culture media, anti-HTLV-I positive pooled sera, anti-HTLV-I negative pooled sera, or each purified antibody in 10% FCS and 10% crude IL-2[16] supplemented RPMI-1640 media, on 24-well plates. The antibody titer of the added anti-HTLV-I antibody-positive pooled sera and those of purified antibodies in the culture media were adjusted to x32 by the immunofluorescence (IF) method. Half of the culture media were changed twice a week, and the cells were cultured for 56 days. Every 7 days after the commencement of culture, part of the cultured cells were collected, washed 3 times with phosphate buffered saline (PBS), and then applied to the glass slide, fixed with acetone, and preserved at -20°.

The expressions of HTLV-I group specific antigen (gag p19) or HTLV-I envelope antigen (env gp46) were examined using an indirect IF method with Gin1417) or purified anti-HTLV-I $gp46^{175-199}$ as the first antibody, and with FITC labeled anti-mouse IgG $F(ab)'_2$ (goat) or with FITC labeled anti-human IgG $F(ab)'_2$ (goat) as the second antibody.

RESULTS

No antibodies against other fractions were recognized in the purified anti-HTLV-I $p19^{100-130}$ and HTLV-I $gp46^{175-199}$ antibodies, both of which were derived from sera of anti-HTLV-I antibody positive pregnant women (Fig. 1).

We could not detect the expression of HTLV-I antigens in PBLs from the pregnant HTLV-I carriers, when the PBLs were cultured in the media with the pooled anti-HTLV-I sero-positive serum, this even on the 56^{th} day after beginning the culture.

Starting from the 14^{th} day after beginning the culture, expressions of both HTLV-I p19 and gp46 antigens were recognized in all 3 specimens from the pregnant HTLV-I carrier PBLs, which were cultured in the media with the pooled anti-HTLV-I sero-negative serum added. Further, the ratio of antigen-positive cells increased as the culture period was prolonged.

In pregnant HTLV-I carrier PBLs cultured with the addition of anti-HTLV-I $p19^{100-130}$ antibody, neither HTLV-I p19 nor gp46 antigens were expressed even on the 56^{th} day (the final day of the experiment). But, in cultured PBLs with the addition of anti-HTLV-I $gp46^{175-199}$ antibody, both HTLV-I p19 and gp46 antigens were expressed at 14^{th} day and later

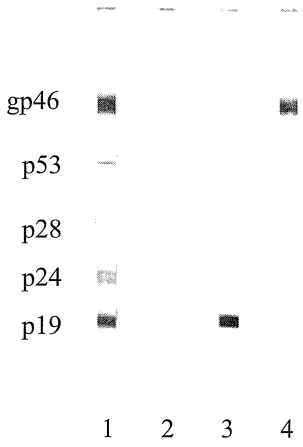

Fig.1. Western blot analysis of fractionated antibody
lane 1 : anti-HTLV-I positive pooled serum
lane 2 : anti-HTLV-I negative pooled serum
lane 3 : purified anti-p19$^{100-130}$
lane 4 : purified anti-gp46$^{175-199}$

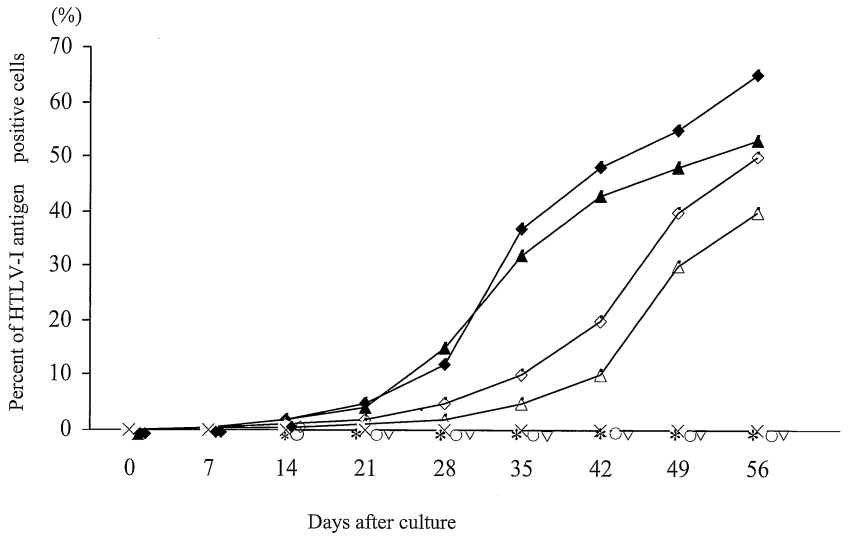

Fig. 2. HTLV-I antigen positive cell rate in healthy carrier 1 serum and purified antibody containing medium
◆p19 positive rate in anti-HTLV-I negative pooled sera containing medium ▲p19 positive rate in anti-gp46$^{175-199}$ containing medium ◇gp46 positive rate in anti-HTLV-I negative pooled sera containing medium △gp46 positive rate in anti-gp46$^{175-199}$ containing medium ×p19 positive rate in anti-HTLV-I positive pooled sera containing medium ○p19 positive rate in anti-p19$^{100-130}$ containing medium ＊gp46 positive rate in anti-p19$^{100-130}$ containing medium ▽gp46 positive rate in anti-HTLV-I positive pooled sera containing medium

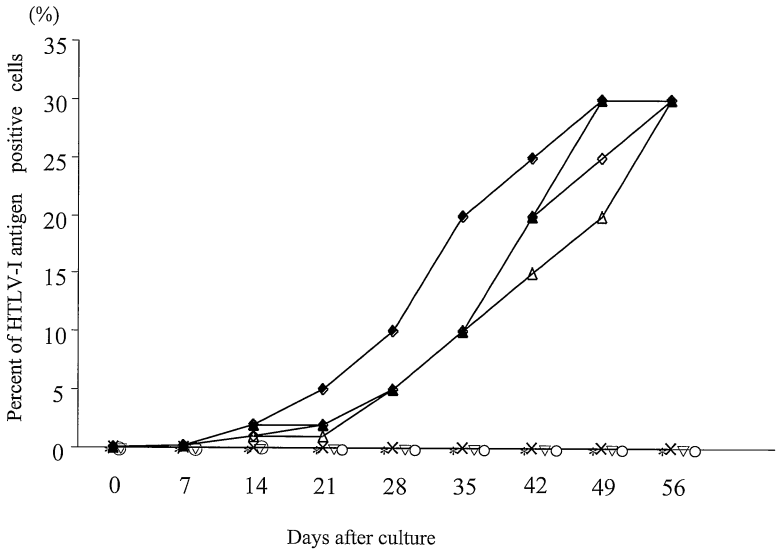

Fig. 3. HTLV-I antigen positive cell rate in healthy carrier 2 serum and fractionated antibody containing medium
◆p19 positive rate in anti-HTLV-I negative pooled sera containing medium ▲p19 positive rate in anti-gp46$^{175\text{-}199}$ containing medium ◇gp46 positive rate in anti-HTLV-I negative pooled sera containing medium △gp46 positive rate in anti-gp46$^{175\text{-}199}$ containing medium ×p19 positive rate in anti-HTLV-I positive pooled sera containing medium ○p19 positive rate in anti-p19$^{100\text{-}130}$ containing medium ＊gp46 positive rate in anti-p19$^{100\text{-}130}$ containing medium ▽gp46 positive rate in anti-HTLV-I positive pooled sera containing medium

of this experiment (Fig. 2, 3, 4).

DISCUSSION

The existence of suppressive effects of the anti-HTLV-I antibody-positive serum on the expression of HTLV-I antigen in cultures has been reported[18], and was also confirmed in our examinations using sera from pregnant anti-HTLV-I antibody-positive women. On the other hand, from an XC cell assay using MT-2 cell line expressing HTLV-I antigen continuously of HTLV-I antigen as a source of infection, it has been revealed that anti-HTLV-I p19$^{100\text{-}130}$ antibodies do not possess an inhibitory action for HTLV-I infection[19]. Further, it has been reported[8,9] that anti-HTLV-I antibody-positive serum exerts inhibitory effects on infections of uninfected cells from persistently infected HTLV-I cell lines. Judging from the past reports[10,13-15] we examined, it can be speculated that the inhibitory action for HTLV-I infection in anti-HTLV-I antibody-positive serum depends heavily on the anti-HTLV-I gp46$^{175\text{-}199}$ antibody.

In examining the intercellular infection of HTLV-I, persistently infected lines that have the expressed HTLV-I antigens are generally utilized as the source of infection. However, it has been shown that HTLV-I antigen is not expressed in vivo in the peripheral PBL of HTLV-I carriers[20].

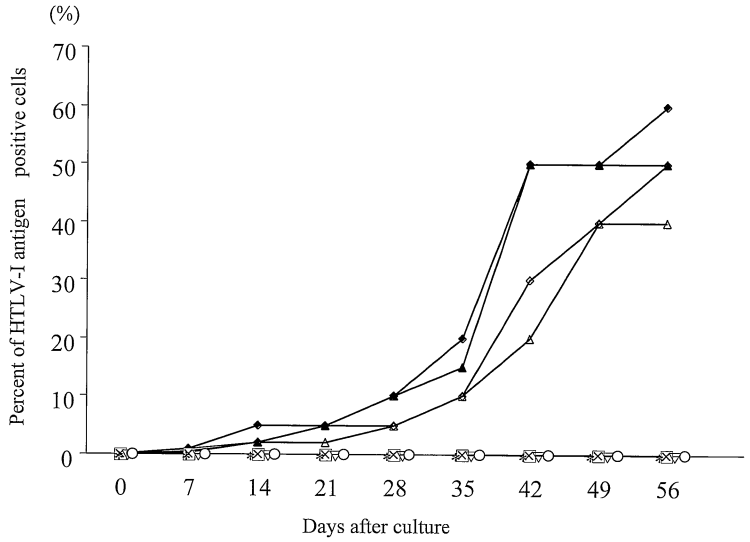

Fig. 4. HTLV-I antigen positive cell rate in healthy carrier 3 serum and fractionated antibody containing medium

◆p19 positive rate in anti-HTLV-I negative pooled sera containing medium ▲p19 positive rate in anti-gp46$^{175-199}$ containing medium ◇gp46 positive rate in anti-HTLV-I negative pooled sera containing medium △gp46 positive rate in anti-gp46$^{175-199}$ containing medium ×p19 positive rate in anti-HTLV-I positive pooled sera containing medium ○p19 positive rate in anti-p19$^{100-130}$ containing medium ＊gp46 positive rate in anti-p19$^{100-130}$ containing medium ▽gp46 positive rate in anti-HTLV-I positive pooled sera containing medium

In our examinations, when anti-HTLV-I antibodies and PBLs were separated from the peripheral blood of pregnant anti-HTLV-I sero-positive women, the preventive effects on the expression of HTLV-I antigens were seen to exist in the anti-HTLV-I p19$^{100-130}$ antibody. However, anti-HTLV-I gp46$^{175-199}$ antibody showed no action to suppress the expression of HTLV-I antigens. This suggests that anti-HTLV-I p19$^{100-130}$ antibody exercise the inhibitory action for HTLV-I infection from HTLV-I carrier's PBLs not expressing HTLV-I antigens, as blood transfusions[21] and in milk-born transmissions from carrier mothers to children[22-24].

On the other hand, from the fact that anti-HTLV-I gp46$^{176-199}$ antibody prevents the transmission of HTLV-I from HTLV-I antigen-positive cell lines[19], the preventive effects in the HTLV-I antigen expressing cells exist in anti-HTLV-I gp46$^{175-199}$ antibody against expressed HTLV-I env antigen.

From a consideration of the above, HTLV-I transmission from HTLV-I carrier's PBLs is prevented by having anti-HTLV-I p19$^{100-130}$ antibody by suppression of the HTLV-I antigens expression.

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