Antibodies reacting to mimotopes of Simian Virus 40 Large T antigen, the viral oncoprotein, in sera from children

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Abstract

Background and purpose: Recent data indicate that Simian virus 40 (SV40) infection appears to be transmitted in human independently from early SV40-contaminated anti-polio vaccines. Serum antibodies against SV40 Large T antigen (Tag) were analyzed in children/adolescents and young adults.

Methods: To investigate antibodies reacting to SV40 Tag epitopes, serum samples (n=812) from children and young adults were analyzed by indirect ELISAs using specific SV40 Tag mimotopes. Mimotopes were synthetic peptides mimicking/corresponding to SV40 Tag epitopes.

Results: In sera (n=412) from healthy children up to 17 years old, IgG antibodies against SV40 Tag mimotopes reached an overall prevalence of 15%. IgM antibodies against SV40 Tag were detected in sera of children 6–8 months old confirming and extending the knowledge that SV40 seroconversion occurs early in life. In children/adolescents affected by different diseases (n= 180) SV40 Tag had a prevalence of 18%, being the difference no significant compared to healthy subjects (n=220; 16%) with the same mean age.

Conclusion: Our immunological data indicate that the SV40 circulates in children and young adults, both in healthy conditions and affected by distinct diseases. The IgM detection in sera from healthy children suggests that the SV40 infection/seroconversion occurs early in life (> 6 months). Our immunological data support the hypothesis that SV40, or a closely related still unknown polyomavirus, infects humans. The SV40 prevalence is lower than common polyomaviruses, such as BKPyV and JCPyV, and other new human polyomaviruses. In addition, our immunological surveillance indicates a lack of association between different diseases, considered herein, and SV40.

Introduction

It has been reported that an unknown proportion of formalin-inactivated anti-poliovirus vaccine lots, belonging to the Salk intramuscular vaccine, were contaminated with small amounts of infectious Simian Virus 40 (SV40). Even a greater amount of infectious SV40 was present in the live oral anti-polio vaccine lots, the Sabin vaccine (Mortimer et al. 1981, Butel 2012). These early SV40-contaminated anti-polio vaccines were administered to millions of children and adults between 1955 and 1963 (Carbone et al. 1997, Barbanti-Brodano et al. 2004, Peden et al. 2008, Butel 2012) as well as in subsequent periods (Cutrone et al. 2005). These contaminations with the adventitious SV40 occurred during the preparations of the vaccines, because the poliovirus strains were grown in primary cell cultures from the kidney of wild monkeys taken from the Indian jungle (Hilleman M, 1960)

It turned out that this monkey is the natural host of SV40 (Payton et al. 2004, Westfall et al. 2008).

Several investigators published that SV40 is a transforming and oncogenic viral agent (Martini et al. 2007, Pipas 2009, Butel 2012). Indeed, SV40 transforms in vitro different animal and human cells of different types, whereas SV40 inoculated by different routes in experimental animals induces cancers of different histotypes, such as brain and bone tumors (Diamandopoulos 1972, Palmiter et al. 1985) lymphoproliferative disorders (Robinson et al. 2006, Butel 2012) and malignant pleural mesothelioma (Carbone et al. 1994). The SV40 transformation and oncogenic capabilities are due to the large T (Tag) and small t (tag) antigens, two early viral proteins acting as activated oncogene products both in cell cultures and in vivo (Barbanti-Brodano et al. 2004, Martini et al. 2007). Like JCPyV in immunosuppressed humans, SV40 is neurotropic and causes PML-like lesions in immunocompromised Rhesus macaques (Horvath et al. 1992, Simon et al. 1999, Kaliyaperumal et al. 2015).

Several studies, carried out mainly by molecular biology methods, such as PCRs (Barbanti-Brodano et al. 2004, Martini et al. 2007) detected SV40 sequences in human tumor specimens of the same histotypes induced in rodents (Klein et al. 2002, Kroczynska et al. 2006, McNees et al. 2009, Bloomfield and Duesberg 2015, Allaman-Pillet et al. 2017). These data indicate that at present SV40

is contagiously transmitted in humans by horizontal infection, independently from the administration of early SV40-contaminated anti-polio vaccines (Barbanti-Brodano et al. 2004). However, some studies reported that SV40-positive results were due to laboratory contaminations with recombinant plasmids carrying SV40 sequences (Lopez-Rios et al. 2004). While it possible that some data were due to laboratory contaminations (Lopez-Rios et al. 2004), it is difficult to think that all investigators obtained false positive results.

In recent investigations, we employed an innovative immunological approach to verify the SV40 footprints in humans. In brief, SV40 antibodies were detected in sera of healthy individuals, at low prevalence, and in sera of patients affected by different cancers, at higher prevalence (Mazzoni et al. 2016, Tognon et al. 2016, Mazzoni et al. 2017, Mazzoni et al. 2017). These results obtained with a highly specific indirect ELISAs with viral capsid protein (VP) mimotopes support and extend previous reported data, i.e. SV40 circulates in humans and it is associated with some human cancers (Klein et al. 2002, Heinsohn et al. 2009, Mazzoni et al. 2012, Mazzoni et al. 2015).

It is worth recalling that the SV40 infections in humans before the administration of contaminated vaccines cannot be excluded. In fact, even now in some Countries humans and monkeys live in close contact, such as in rural Indian villages and temples. It has been proved that SV40 can infect humans by crossing easily the specie specific barrier (Payton et al. 2004).

The detection of SV40 antibodies in human sera is an important parameter with which to measure the spread of SV40 infection in humans and to verify its association with human pathologies.

Few studies were carried out on SV40 seroprevalence in pediatric populations (Kean et al. 2009, Cason et al. 2018). A recent work (Taronna et al. 2013) reported SV40 antibodies against its viral protein antigens (VPs 1-2-3) with a prevalence of 16% in sera of healthy children and adolescents. More recently, we set up and developed an indirect ELISA with mimotopes representing specific SV40 Tag epitopes to detect antibodies against the viral oncoprotein Tag (Tognon et al. 2016). These innovative immunological approaches may circumvent the criticisms arose with the fore mentioned use of PCR methods and problems related to the employment of immunological data obtained with

enzyme immunoassays (EIAs/ELISA) with the viral-like particles (VLP) or the recombinant VP1 as antigens. Indeed, VLPs had the disadvantage to cross-react, to some extends, with the other highly homologue polyomavirus VP 1 antigens (Viscidi et al. 2003, Shah et al. 2004).

The objective of the present study was to evaluate the presence of SV40 Tag antibodies by indirect ELISA with specific mimotopes in sera of different cohorts of children, adolescents and young adults.

Materials and Methods

Sera samples

Serum samples (n=632) from healthy children, adolescent and young adults from 1 month up to 33 years old were collected in the Clinical Laboratory of the University Hospital of Ferrara, Italy. These healthy subjects were analyzed for a routine check-up analysis, whereas the hospital records indicated that they were in good health at the time. Sera were taken from discarded laboratory specimens after routine analyses before the incineration. Anonymously collected sera were coded indicating the age and gender only. In this investigation, another serum collection represented by samples from children and adolescents affected by different diseases (n=180) was analyzed. These sera were from the Institute for Maternal and Child Health Hospital, Trieste, Italy. Sera (n=72; mean age 15 yrs old) were from autoimmune disease affected patients. These patients were affected by IgA anti transglutaminase value alteration (n= 27), juvenile idiopathic arthritis (n= 17), Chron's disease (n=16); ulcerative colitis (n=3), antinuclear antibody positive-test (n=2), transplanted (n=2), chronic infantile neurological cutaneous articular syndrome (n=1), chronic glomerulonephritis (n=1); Behcet's disease (n=1), pars planitis (n=1), and scleroderma cutaneous (n=1). In addition, serum samples (n=108) from children and adolescents affected by other distinct diseases were collected: immunodeficiency (n=44, mean age 13yrs), acute myeloid leukemia, juvenile myelomonocytic leukemia and acute lymphoblastic leukemia (n=42, mean age 12 yrs), inflammatory bowel disease (n=9, mean age 13 yrs) and periodic fever (n=13, mean age 14 yrs). Parents of <18 years old subjects/patients, healthy subjects and patients > 18 years old, affected by different diseases gave their written informed consent. The County Ethical Committee of Ferrara approved the study.

Indirect ELISA

The method of Indirect ELISA has been described previously (Mazzoni et al. 2016, Tognon et al. 2016, Mazzoni et al. 2017). SV40 Tag peptides, known as A and D, respectively, which are encoded

by the viral Tag early gene were designed as described (Tognon et al. 2016). The a.a. sequences of the two Tag A and D mimotopes/antigen are as follows:

SV40 Tag A: NH2-G S F Q A P Q S S Q S V H D H N Q P Y H I-COOH (Tag a.a. 669–689, 21 a.a).

SV40 Tag D: NH2-H E T G I D S Q S Q G S F Q A P Q S S Q S V H D-COOH (Tag a.a. 659–682, 24 a.a).

Tag synthetic peptides A and D were employed in indirect ELISAs because reacting specifically with a hyperimmune serum of a rabbit immunized with the purified SV40 Tag protein (positive control serum) and with SV40 VP-positive human sera, tested earlier (Corallini et al. 2012, Tognon et al. 2016). The two selected peptides Tag A and D did not cross-react with BKPyV and JCPyV hyperimmune sera employed as negative controls. An additional human negative peptide, used as control, was the neuropeptide S (hNPS), non-liked to SV40, a.a. sequence SFRNGVGTGMKKTSFQRAKS. The synthetic peptides were from the UFPeptides s.r.l., Ferrara, Italy. *Peptide Coating.* ELISA plates (Nunc-immuno plate PolySorp, CelBio, Milan) were coated with 5 µg Tag A and D mimotopes diluted in 100 µl of Coating Buffer 1x, pH 9.6 (Candor Bioscience, Wangen, Germany) and incubated overnight (16–24 h) at 4 °C. *Blocking Phase.* Plates were washed three times with Washing Buffer (Candor Bioscience, Wangen, Germany). Blocking was performed with the blocking solution containing casein (200 µl/well) (Candor Bioscience, Wangen, Germany) at 37°C for 90 min. *Primary Antibody.* Plates were rinsed three times with the washing buffer (Candor Bioscience, Wangen, Germany) using a washing apparatus (Thermo Electron Corp., model Well wash 4MK2, Vantaa, Finland). Human serum samples from children and adolescents, diluted in Low Cross-Buffer pH 7.2 (Candor Bioscience, Wangen, Germany) at 1:20, were added to the plate. Different wells were covered with diluted sera; (i) the positive control represented by the immune rabbit serum containing anti-SV40 Tag Ab; (ii) the negative controls were immune sera with anti-BKPyV and anti-JCPyV Ab and (iii) three human serum samples tested SV40 Tag-negative in earlier studies (Mazzoni et al. 2016, Tognon et al. 2016, Mazzoni et al. 2017). Additional control was 100 µl of Low Cross-Buffer pH 7.2 void of both primary and secondary Ab . The plate was incubated at

37°C for 90 min. *Secondary Antibody Addition.* After 90 min of incubation, a triple rinsing cycle was repeated and then the secondary antibody solution was added to each well. The anti-human or goat anti-rabbit IgG heavy (H) and light (L) chain-specific peroxidase conjugate (dilution 1:10,000, Calbiochem-Merck, Darmstadt, Germany) in Low Cross-Buffer (Candor Bioscience, Wangen, Germany) was added to all wells (100 µl/well) and incubated at 37° for 90 min. *Optical Density (OD) Reading*. Plates were washed three times with Washing Buffer (Candor Bioscience, Wangen, Germany) and then treated with 100 µl of 2,2′-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid (ABTS) solution (Sigma-Aldrich, Milan, Italy), which reacted with the peroxidase enzyme to yield the color reaction if immune complexes formed by the presence of specific Ab, which bound to the SV40 Tag synthetic peptide/epitopes/mimotopes. The plate was read spectrophotometrically a wavelength (λ) 405 nm (Thermo Electron Corp., model Multiskan EX, Vantaa, Finland).

Cut-off Determination. The method of cut-off values determination has been described previously. Briefly, cut-off values were determined for each assay using the OD reading of the three negative control sera, which were added to the SD and multiplied three times (+3 SD). The three SV40-negative control sera were selected from those below the cut-off value determined with second-degree polynomial regression by plotting the ranked net OD individual value for each peptide. The OD representation was obtained from a second-degree polynomial regression for Tag A and D mimotopes, as reported before for BKPyV and MCPyV VLPs (24, 25). Our representations show an inflection point for peptide A and peptide D at 0.19 and 0.18, respectively. Immune serum samples were considered SV40 Tag-positive when reacting to both peptides A and D, in three replica ELISA experiments carried out by three different operators.

Neutralization of SV40 Infectivity in Permissive Cells by Serum Ab from healthy children and adolescents.

CV-1 monkey kidney cells, which are cells permissive to SV40 infection, were employed for the neutralization assay in vitro. The SV40 infectivity was scored as a reduction of the number of plaque

forming units (PFU) in CV-1 monolayers. The neutralization of the SV40 infectivity was assayed by mixing the human serum under analysis, diluted at 1:20 in phosphate buffered saline (PBS), with 5×10^4 PFU of SV40; then, the solution was incubated at 37°C for 30 min. The solution was layered on the CV-1 monolayer for 2 h at 37°C. Then, the inoculum was discarded from the cells, whereas the monolayer was rinsed three times with DMEM. Each sample was analyzed twice. The controls were as follows: (i) CV-1 with PBS; (ii) SV40 with PBS; (iii) SV40 with an immune human sample, the positive control; (iv) SV40 with a non-immune serum, the negative control. The appearance of the SV40 cytopathic effect (CPE) had inspected in CV-1 infected monolayers by a light microscope for 21 days. The neutralization assay was performed in triplicate, while the neutralizing activity of the serum under analysis was checked by the inhibition of SV40 CPE.

Statistical Analysis

The prevalence of SV40-positive serum samples from healthy children and adolescent with different age was determined using chi-square with Yates' correction. The serologic profile of serum antibody reactivity to SV40 Tag mimotopes was statistically analyzed using the Anova tests. All computational analyses were performed with Prism 6.0 (GraphPad software, San Diego, CA, USA). For all tests, P was considered to be statistically significant when $P < 0.05$.

Results

Serum IgG antibodies reacting to SV40 Tag mimotopes in healthy children and adolescents.

Indirect ELISA with SV40 Tag peptide A, was employed to test serum samples, which had been diluted at 1/20. Sera were from healthy children and adolescent 1 month–17 years old (Table 1). The SV40 Tag prevalence was stratified by age: <1 month-1 year; 1.1 year-10 years old and 11-17 years old (Table 1). In addition the SV40 Tag prevalence in the cohort of children of <1 month-1 years old, was stratified as < 1 month–4 months old and 4.1 months –1.1 years old. The SV40 Tag prevalence in the cohort of children and adolescents 1.1-10 yrs old was stratified as 1.1-3 years old; 3.1-6 years old and 7-10 yrs old (Table 1).

Healthy subjects (HS) reacted to mimotope A with an overall prevalence of 16%. The different prevalence among the three cohorts is not statistically significant (P>0.05) (Tables 1).

Then, sera were assayed by indirect ELISAs with SV40 Tag mimotope D. The prevalence of IgG antibodies from healthy children and adolescents reacting to this antigen was 17%. The different prevalence among age cohorts of children is not statistically significant (Table 1).

In our ELISA only serum samples reacting with both synthetic peptides A and D were accepted as SV40 Tag-positive.

The prevalence has an increasing trend being in children up to 1 year old 13% (12/95), in those 1-10 years old 14% (32/226), whereas it was 18% (16/91) in young individuals 11-17 years old. Overall, in young individuals aged 1 month-17 ys old the prevalence of antibodies against SV40 Tag mimotopes was 15% (60/412). (Table 1). The OD readings of serum samples, stratified by age, <1 month-17 years old, and their serologic profiles are shown in Figure 1. High levels of antibodies against both SV40 Tag peptides A+D were observed in children aged 4–12 months *vs* children aged 0-4 months, 1.1-3 yrs old, 3.1-6 yrs old, (*P<0.05) and *vs* children of 7-10 yrs old (***P<0.000). In addition high levels of antibodies were observed in children aged 7-10 yrs *vs* adolescent aged 11-17 yrs (P<0.05) (Figure 1).

Serum IgM antibodies against SV40 Tag

To verify the age of SV40 Tag seroconversion five sera from children aged 2–8 months old were analyzed. To this purpose, IgM antibodies were investigated by indirect ELISAs with SV40 Tag A and D peptides. It turned out that two samples from healthy children, 2–4 months old, were IgM negative, while the other three sera from children 6-8 months old tested IgM positive. This result indicates that SV40 seroconversion occurred in children > 6 months old (Table 2).

Serum IgG antibodies against SV40 Tag mimotopes in young adults and in young patients affected by different diseases.

Serum samples from healthy subjects (n=220) and patients (n=180) affected by different diseases were analyzed by indirect ELISAs with SV40 Tag mimotopes. Sera were from patients affected by autoimmune diseases, immunodeficency; acute myeloid leukemia, juvenile myelomonocytic leukemia and acute lymphoblastic leukemia, inflammatory bowel disease, periodic fever (Table 3). Overall, in healthy children, adolescent and young adults aged 2-33 years old the prevalence of antibodies against SV40 Tag mimotopes was 16% (35/220), while in children, adolescents and young adult patients aged 2-33 years old the prevalence was 18% (33/180) (Table 3). The prevalence of SV40 Tag antibodies in the five different cohorts of young patients was 16% (12/72) in autoimmune disease patients, 20% (9/44) in patients affected by immunodeficiency disease, 17% (7/42) in acute myeloid leukemia, juvenile myelomonocytic leukemia and acute lymphoblastic leukemia, 22% (2/9), in inflammatory bowel disease and 23% (3/13) in periodic fever. The different prevalence of SV40 Tag among patients, was not significant (P>0.05) (Table 3) nor it was the different prevalence in patients 18% (33/180) compared to healthy subjects 16% (35/220) (P>0.05) (Table 3). The prevalence of SV40 Tag antibodies in autoimmune disease was stratified in different subtypes (Table 4).The prevalence in AD patients affected by distinct autoimmune disease were not statistically significant (P>0.05) (Table 4).

Neutralization of SV40 infectivity in permissive CV-1 monkey cells

To verify the neutralization activity of SV40 Tag immune sera from healthy children and young patients an inhibition test was performed (Mazzoni et al. 2014). SV40 immune sera (n=4) from healthy subjects and children affected by inflammatory diseases (n = 2) with an OD (range OD = 0.193–0.876) were challenged to inhibit the SV40 CPE in CV 1 cells infected by SV40. In this neutralization assay, together with SV40-positive sera, two additional SV40-negative sera (range OD = 0.071–0.111) were added, as control,. SV40 CPE was hampered or abolished indicating that tested sera carried neutralizing Ab against SV40 (Figure 2). This result strongly suggests that a SV40 infection occurred in young individuals/patients, tested SV40-positive.

Discussion

In our study serum samples from children, adolescents and young adults were assayed with an innovative indirect ELISA using as mimotopes specific SV40 Tag synthetic peptides. Sera were from different cohorts of healthy subjects and patients affected by distinct pathologies. Our investigation, which tested the presence of serum IgG antibodies against SV40 Tag, the viral oncoprotein, is the first study regarding different cohorts of children reported in this field. Our immunological assay employing specific SV40 Tag mimotopes as synthetic peptides corresponding to the viral oncoprotein antigens allowed us to estimate the seroprevalence of serum antibodies in the analyzed cohorts.

The results of this investigation indicate that the overall prevalence of IgG antibodies against SV40 Tag in sera from healthy children and young patients affected by different pathologies was 16% and 18%, respectively.

The serum samples stratified by age of individuals/patients did not show a different prevalence of antibodies reacting to SV40 Tag. The prevalence has an increasing trend being in children up to 1 year old 13%, in those 1-10 years old 14%, whereas it was 18% in young individuals 11-17 years old. It has been reported that SV40 could be transmitted through contact in the familiar/home environment, as well as in other communities, such as schools. In previous studies, SV40 DNA

sequences were detected in different specimens such as blood, stool and urine from children and adults. These data indicate that the SV40 spread may occur by distinct routes of transmission in the general population. Our immunological data confirm and extend results previously obtained by molecular biology methods

It is interesting to note that sera from children 4-12 months old show antibodies against SV40 Tag with higher OD values than in other age groups of HS. These results were obtained for the Tag peptide A, Tag peptide D and pooling the OD data of Tag peptides A+D (Figure 1). It is possible that the ability of children to respond to SV40 Tag antigens with specific antibodies against this viral oncoprotein increases overtime because of continuous antigenic stimulations. In addition, indirect ELISAs revealed the presence of IgM antibodies against SV40 Tag in serum samples taken from children 6-8 months old.

In sera (n=180) from young patients affected by distinct diseases analyzed by indirect ELISAs, the prevalence of serum IgG antibodies reacting to SV40 T antigen was 18% (33/180). It turned out that the prevalence of SV40 Tag antibodies detected in this cohort of patients did not differ significantly from HS (16%; $P>0.05$).

This result is important because indicates that the diseases considered in this study do not influence the antibody response to SV40 Tag.

Autoimmune diseases may be induced via molecular mimicry, particularly from infectious agents, but host factors and confounding host responses may modulate the disease susceptibility and natural history (Rodriguez et al. 2018). In our study, a large sample size of sera from patients affected by different types of autoimmune diseases were assayed by indirect ELISAs to verify the prevalence of SV40 Tag antibodies. Sera from distinct autoimmune diseases did not show a significant different prevalence of SV40 Tag antibodies. The low SV40 seroprevalence revealed in this study suggests that SV40 is less transmissible than other human polyomaviruses (HPyV), such as BKPyV, JCPyV and Merkel cell polyomavirus (MCPyV) that infect most of healthy subjects, approx. 80%. These polyomaviruses have the potential of causing severe organ damage or malignant transformation,

especially in individuals with weakened immunity who are unable to mount or regain endogenous T-cell responses, as a result of underlying leukemia or iatrogenic immunosuppression in autoimmunity, bone marrow and solid organ transplant settings (Davies and Muranski 2017). The relationship of recurrence infection and immunodeficiency has been studies, but at present is not fully understood. Our data on the prevalence of SV40 Tag antibodies in healthy young subjects indicates that this polyomavirus infects the human population since the childhood, while the prevalence of SV40 Tag antibodies in young patients suggests that SV40 is not associated with diseases analyzed in this investigation.

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Figures' legends

Figure 1. Serologic profile of children and adolescent serum antibody reactivity to SV40 large T antigen mimotopes Tag A (A), Tag D (B) and Tag A+D (C).

Immunologic data are from healthy subjects (HS). Results are presented as values of OD readings at λ 405 nm for serum samples diluted 1:20 and assayed in indirect ELISA. In this scatter dot plotting, each plot represents the dispersion of individual sample OD values to a mean level, indicated by the long horizontal line inside the scatter with standard error of the mean (SEM) marked by short horizontal lines for each age group. The OD readings of serum samples were stratified by age: 0–4 months (mo), 4–12 mo, 1.1– 3 years (yrs), 3.1-6 yrs, 7-10 yrs, and 11-17 yrs. Data were analyzed with one way Anova analysis and Newman- Keuls Multiple Comparison test (OD mean, 95% CI). (A) High levels of antibodies against SV40 Tag A in children aged 4–12 months (0.25 OD, 95% CI = 0.18-0.32) *vs*. children aged 3.1–6 yrs (0.13 OD, 95% CI =0.10-0.17), *P < 0.05, and *vs*. children aged 7–10 yrs (0.13 OD, 95% CI =0.11-0.14),**P < 0.001). (B) High levels of antibodies against children aged 4–12 months (0.22 OD, 95% CI =0.16-0.28) *vs* children aged 7–10 yrs (0.13 OD, 95% CI = 0.11-0.14), (*P<0.05). (C) High levels of antibodies against both SV40 Tag peptides Tag A+D were observed in children aged 4–12 months (0.24 OD, 95% CI =0.19-0.28), *vs* children aged 0-4 months (0.16 OD, 95% CI = 0.12-0.19) *P<0.05, *vs* children aged 1.1-3 yrs (0.15 OD, 95% CI =0.12-0.19) *P<0.05, *vs* childrens aged 3.1-6 yrs, (0.15 OD, 95% CI =0.12-0.18) *P<0.05 and vs children aged 7-10 yrs, (0.12 OD, 95% CI = 0.11-0.13) ***P<0.0001. High levels of antibodies were observed in children aged 7-10 yrs (0.12 OD, 95% CI = 0.11-0.13) *vs* adolescent aged 11-17 yrs (0.18 OD, 95% CI = 0.15-0.21) P<0.05.

Figure 2. Inhibition of Simian virus 40 (SV40) cytopathic effect (CPE) in CV-1 infected cells by human immune sera. Inhibition of SV40 CPE in infected cells by human serum samples of children, adolescents and young adults, healthy subjects and autoimmune disease patients. (A) Negative control represented by uninfected CV-1 cells. (B) Positive control represented by the CPE induced by SV40 in CV-1 infected cells. (C, D) Serum samples of autoimmune disease: these sera in part inhibited the SV40 CPE. (E–F) SV40-positive sera completely inhibited SV40 CPE.