Antibody-Enhanced Infection by HIV-1 Via Fc Receptor–Mediated Entry

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Monocytes and macrophages, which may play a central role in the pathogenesis of infection with human immunodeficiency virus type 1 (HIV-1), express the CD4 molecule and Fc receptors (FcR) for immunoglobulin G (IgG). To explore the possibility that FcR mediate HIV-1 infection of monocytes, studies were conducted with the human monocytic cell line U937. These cells were exposed to HIV-1 complexed with various concentrations of serum from HIV-1 antibody-positive individuals and monitored for HIV-1 replication. Serum samples from antibody-negative normal individuals did not affect virus yields. High concentrations of antibody-positive sera showed virus-neutralizing activity; however, cells infected with HIV-1 in the presence of antibody-positive sera at subneutralizing concentrations significantly enhanced virus replication. This infection enhancement was blocked by heat-aggregated γ-globulin. Moreover, the IgG fraction from an HIV-1 antibody-positive serum enhanced HIV-1 infection at the same serum dilution equivalents. In contrast, IgG-F(ab')$_2$ did not enhance HIV-1 infection but showed neutralizing activity with HIV-1. These results are compatible with the concept of FcR-mediated infection enhancement and suggest that this immunological response to HIV-1, instead of protecting the host, potentially facilitates the infection.

THERE IS EVIDENCE THAT T-LYMPHOCYTE dysfunction and depletion of T helper-inducer cells in patients with acquired immunodeficiency syndrome (AIDS) may be due to the selective tropism of HIV-1 for the CD4 molecule (*1*). However, the slow development of the disease and the low frequency of cells expressing HIV-1 messenger RNA among circulating lymphocytes (*2*) has led to the suggestion that cells of the monocyte-macrophage lineage, including glial cells in the brain (*3*) and follicular dendritic cells in lymph nodes (*4*), may serve as reservoirs for virus dissemination and persistence (*5*). Latent infection of monocytes had been demonstrated in visna virus infection (*6*).

Human monocytes as well as monocytic cell lines can be infected with HIV-1 (*5*) and these cells have the CD4 receptor (*7*). Nevertheless, it is possible that the CD4 molecule is not the only binding site for virus entry. Some glial cells are susceptible to HIV-1 infection but do not appear to express CD4 (*8*). It has been demonstrated that HIV-1 penetrates T cells via direct fusion with the plasma membrane (*9*), but this may not be the sole mechanism of HIV-1 entry into monocytes. Theoretically, HIV-1 might enter mononuclear phagocytes by three mechanisms: (i) nonspecific phagocytosis, (ii) CD4-mediated binding of HIV-1 to the cell membrane and subsequent fusion, and (iii) receptor-mediated endocytosis of antibodies to HIV-1 complexed with HIV-1, that is, antibody-dependent virus entry via Fc receptors (FcR) for immunoglobulin G (IgG) or complement 3b (C3b) receptors (CR1). Antibody-dependent enhancement of infection occurs in other virus systems, such as in flavivirus infections (*10–12*). This opsonic phenomenon occurs when FcR-bearing cells are infected with virus in the presence of antibodies at subneutralizing concentrations. We show here that HIV-1 entry into monocytic cells is mediated by the FcR for IgG, which indicates that FcR-bearing cells have a role in the pathogenesis of immunodeficiency in HIV-1 infections.

Three classes of FcR for IgG have been charaterized on human cells (*13*). Monocytes bear two of the three: FcRI and FcRII, which are the high- and low-affinity receptors, respectively, for human IgG1 or IgG3. In these experiments we used the human histiocytic lymphoma–derived cell line U937, which has monocyte characteristics and bears FcRI, FcRII, and CD4 receptors (*14*). Serum samples tested were obtained from patients with HIV-1 infection (Walter Reed stages I or II) (*15*) who were seropositive for HIV-1 as determined by the immunoblot technique. Serum samples from healthy blood donors without antibodies to HIV-1 were also used. The H9/HTLV-IIIB cell line (*16*) was grown in RPMI 1640 medium supplemented with 20% fetal bovine serum (FBS) and antibiotics. Culture supernatants were harvested and titrated on C8166 cells (*17*) by using a median tissue culture infectious dose ($TCID_{50}$) assay that indicates the dilution that results in syncytia formation in 50% of eight replicate cultures per dilution. Infection of U937 cells was monitored for HIV-1 antigen-positive cells in an indirect immunofluorescene assay by microscopy and for p24 antigen in culture supernatants with an HIV-1 capture enzyme-linked immunosorbent assay (ELISA) (Du Pont) (*18*).

Cells that were HIV-1 antigen positive were first detected on day 5. The number of infected cells increased rapidly between days 7 and 10, when all cells became infected (Fig. 1A). The percentage of immunofluorescence-positive cells depended on the concentrations of antiserum mixed with virus before the cells were inoculated. The diversity probably is the result of the differences in the number of cells originally infected at inoculation with a low multiplicity of infection (MOI) of 0.01 and subsequent dissemination of HIV-1. From days 5 to 10, the percentage of immunofluorescence-positive cells infected with HIV-1 in the presence of a low dilution of antiserum was significantly lower than for the cells infected with virus alone, suggesting the effect of neutralizing antibodies. In contrast, the percentage of cells infected with HIV-1 in the presence of high dilutions of antiserum significantly exceeded the percentage of cells infected by virus alone.

A study of the kinetics of the release of p24 into the supernatants of U937 cell cultures showed that antigen was first detected in supernatants on days 2 to 3; the yield increased in a logarithmic manner (Fig. 1B). These kinetics were compatible with the results of the immunofluorescence analysis. On days 7 to 9, the amount of p24 produced by virus preincubated with a low dilution of antibody was significantly lower than virus alone, implying a neutralizing effect. The production of p24 by cells infected with virus mixed with high dilutions of serum were significantly higher, and indicated enhancement of infection in the presence of antiserum beyond the neutralization end point. It was conceivable that binding of immune complexes to FcR may have stimulated cell growth nonspecifically, and this could result in an increase in virus replication. However, the number of viable cells (Fig. 1C) eliminates this possibility because cell growth at high serum dilutions that enhanced infection was comparatively low.

We next determined virus yields in the supernatants on days 7 or 8, and examined normal sera as well as several other sera with antibodies to HIV-1 for enhancement activity (Table 1). Antibody-negative sera did not affect virus yields and did not enhance virus infection; however, four of five antibody-positive sera showed enhancement activity at the higher serum dilutions, between

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10^{-3} and 10^{-6}, ranging from 1.5 to 2.2 as well as demonstrating neutralizing activity at lower dilutions. In one of the sera, no enhancement was detected even at further dilutions although remarkable neutralizing activity was observed at lower dilutions.

To determine whether FcR are responsible for the enhancement of infection in the presence of antibodies, we attempted to block FcR with heat-aggregated normal human γ-globulin (AHG), which is a high-affinity ligand for FcR (*19*) (Table 2). As expected, after mixing with antibody-positive serum, virus yields were approximately doubled, but the addition of AHG resulted in yields similar to those of the control, that is, HIV-1 plus AHG without antisera. These observations strongly suggest that HIV-1 complexed with HIV-1 antibody enters mononuclear phagocytes via FcR, in the absence of complement. We also tested the effects of purified IgG and IgG-$F(ab')_2$ obtained from serum that was HIV-1 antibody positive (*20*) and found that the IgG antibody to HIV-1 neutralized the virus at low dilution and enhanced HIV-1 infection at serum dilution equivalents from 10^{-4} through 10^{-6} (Table 3). In contrast, IgG-$F(ab')_2$ did not enhance HIV-1 infection at any dilution and possessed neutralizing activity to HIV-1. The neutralization seen at low IgG dilutions may be a combination of neutralizing activity and enhancing activity. Thus, the enhancement of HIV-1 infection is mediated by IgG and requires the Fc portion of IgG.

Infection enhancement mediated by antibody has been described for a number of flaviviruses including dengue virus, West Nile virus, yellow fever virus, and Japanese encephalitis virus, as well as alphavirus, poxvirus, bunyavirus, reovirus, rhabdovirus, lactic dehydrogenase virus, and influenza virus in vitro (*10, 21*). The observation of enhanced viremia during dengue virus infection of rhesus monkeys after administration of antibodies and epidemiological studies in which higher rates of severe dengue virus infections were found in children who had been previously infected with dengue virus support an in vivo role for immune enhancement (*22*).

It is important to compare the enhancement of infection we now report for HIV-1

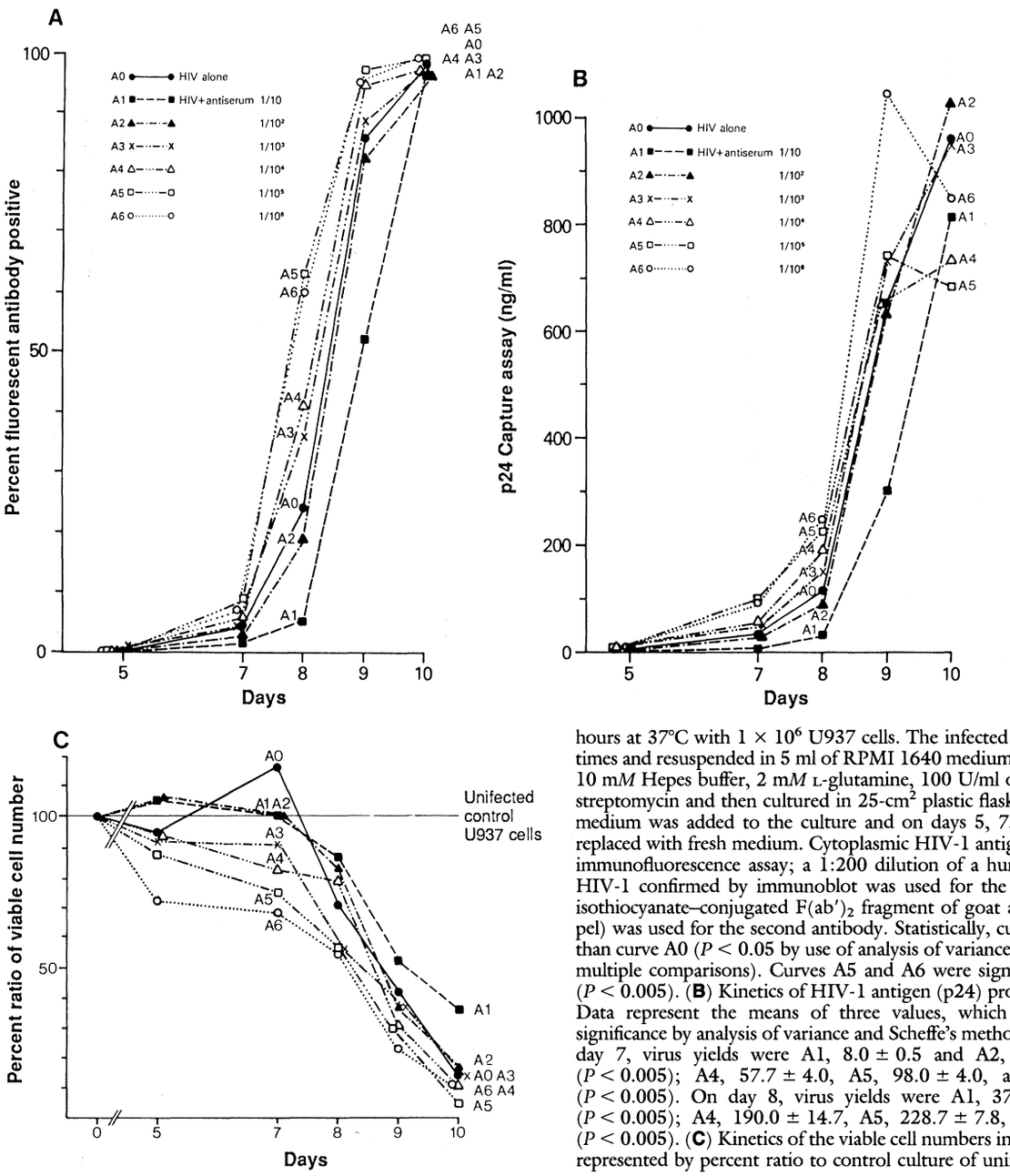

Fig. 1. (**A**) Kinetics of HIV-1 antigen-positive cells in cultures of U937 cells. An HIV-1 inoculum (1 ml) containing 1×10^4 $TCID_{50}$ in RPMI 1640 medium and 2% FBS was mixed with a 1-ml portion of heat-inactivated (56°C for 30 min) HIV-1 antibody-positive serum serially diluted tenfold from 10^{-1} to 10^{-6} in RPMI 1640 medium and incubated for 2 hours at 4°C. These virus-serum mixtures were then incubated for 2 hours at 37°C with 1×10^6 U937 cells. The infected U937 cells were washed three times and resuspended in 5 ml of RPMI 1640 medium supplemented with 10% FBS, 10 m*M* Hepes buffer, 2 m*M* L-glutamine, 100 U/ml of penicillin, and 100 μg/ml of streptomycin and then cultured in 25-cm^2 plastic flasks. On day 3, 5 ml of the same medium was added to the culture and on days 5, 7, and 9, 5 ml of medium was replaced with fresh medium. Cytoplasmic HIV-1 antigen was detected by an indirect immunofluorescence assay; a 1:200 dilution of a human serum with antibodies to HIV-1 confirmed by immunoblot was used for the first antibody and fluorescein isothiocyanate–conjugated $F(ab')_2$ fragment of goat antibody to human IgG (Cappel) was used for the second antibody. Statistically, curve A1 was significantly lower than curve A0 ($P < 0.05$ by use of analysis of variance and the Bonferroni method of multiple comparisons). Curves A5 and A6 were significantly higher than curve A0 ($P < 0.005$). (**B**) Kinetics of HIV-1 antigen (p24) production by U937 cell cultures. Data represent the means of three values, which were analyzed for statistical significance by analysis of variance and Scheffe's method of multiple comparisons. On day 7, virus yields were A1, 8.0 ± 0.5 and A2, 29.3 ± 2.9 < A0 38.0 ± 3.0 ($P < 0.005$); A4, 57.7 ± 4.0, A5, 98.0 ± 4.0, and A6, 87.3 ± 11.0 > A0 ($P < 0.005$). On day 8, virus yields were A1, 37.7 ± 5.0 < A0, 117.3 ± 10.1 ($P < 0.005$); A4, 190.0 ± 14.7, A5, 228.7 ± 7.8, and A6, 254.3 ± 11.6 > A0 ($P < 0.005$). (**C**) Kinetics of the viable cell numbers in cultures of infected U937 cells represented by percent ratio to control culture of uninfected U937 cells.

to that reported for flaviviruses such as dengue. Dengue virus yields are reported to be enhanced from 2- to 10,000-fold in the presence of antibodies, depending on the strain of virus, cell type, and antibody used (*10*). In experiments that were performed in our laboratory with dengue virus and antibody in U937 cells, we found from a four- to tenfold enhancement of infection by antibody similar to those reported here with HIV-1 (*23*). One of the important differences between dengue and HIV-1 infections in vivo is that dengue is an acute illness that lasts from several days to weeks, whereas HIV-1 infections are chronic and progressive. Thus, although antibody-enhanced infection of dengue virus may result in a greater yield of progeny virus compared to HIV-1 in short-term in vitro assays, the chronic, progressive nature of HIV-1 infections suggests that multiple, ongoing cycles of infection occur in vivo amplified by enhanced infection.

The complete mechanism of FcR-mediated infection of cells by viruses has not been elucidated. Antibody-opsonized West Nile virus was reported to have increased binding to the cell surface and appeared to be internalized via receptor-mediated endocytosis (*24*). The following entry pathway of HIV-1 antibody complexes may be postulated from receptor-mediated endocytosis characterized for some enveloped viruses (*25*); the antibody-opsonized HIV-1 particles that are bound to FcR on the cell surface are internalized and enter the endosome, where the receptor-ligand complex dissociates at low *p*H and virus may penetrate into the cytosol.

Recently enhancement of HIV-1 infection of a transformed T cell line was reported with the use of serum factors and was suggested to be complement-mediated (*26*).

Table 1. Infection enhancement activity in HIV-1 seropositive sera. The protocol was as in Fig. 1. Results were obtained from five separate experiments. Each of the experiments was carried out with antibody-negative normal human sera as well as antibody-positive sera, and the culture supernatants were harvested for p24 assays on days 7 or 8. The numbers in parentheses show the enhancement index as compared to the control of HIV-1 alone. The results of the normal serum are those of a representative serum that was one of the five tested antibody-negative sera. This normal serum at serial dilutions did not affect virus yields. Enhancement indices for this normal control serum were 0.95 ± 0.059 (mean ± SD) and the 95% confidence limits of the index values were 0.83 to 1.06. The other four normal sera also did not enhance virus yields, and those limits were less than 1.20; accordingly an enhancement index exceeding 1.20 was regarded as positive. The bold-faced numbers indicate positive enhancement. Four of five antibody-positive sera showed enhancement activity at the higher serum dilutions. In experiments not presented, antibody-positive sera, but not antibody-negative sera, reproducibly enhanced infection.

HIV + serum concentration	p24 (ng/ml)					
	Normal serum	HIV-1 seropositive sera				
		1	2	3	4	5
0*	185.3 (1.0)	117.5 (1.0)	199.5 (1.0)	264.2 (1.0)	95.9 (1.0)	180.8 (1.0)
10^{-1}	173.0 (0.93)	37.7 (0.32)	31.1 (0.16)	84.6 (0.32)	5.9 (0.06)	25.8 (0.14)
10^{-2}	178.4 (0.96)	89.1 (0.76)	86.6 (0.43)	203.1 (0.77)	49.5 (0.52)	103.3 (0.57)
10^{-3}	194.9 (1.05)	**153.3** (1.30)	152.4 (0.76)	228.4 (0.86)	**135.2** (1.41)	102.0 (0.56)
10^{-4}	164.7 (0.89)	**190.0** (1.62)	**267.8** (1.34)	**328.3** (1.24)	**174.1**† (1.82)	130.4 (0.72)
10^{-5}	165.6 (0.89)	**228.8** (1.94)	**326.5**† (1.64)	**400.0**† (1.51)	93.6 (0.98)	127.9 (0.71)
10^{-6}		**254.2**† (2.16)	**243.2** (1.22)	**373.5** (1.41)	72.2 (0.75)	188.2 (1.04)

*HIV alone. †Peak level of enhancement.

Table 2. Effect of blocking of FcR on enhancement of HIV-1 infection. One milliliter of an HIV-1 inoculum containing 1×10^4 $TCID_{50}$ was mixed with 1 ml of RPMI 1640 medium or with 1 ml of heat-inactivated antibody-positive serum diluted $1:10^5$ in RPMI 1640 medium and incubated for 2 hours at 4°C. These mixtures were then incubated for 2 hours at 37°C with 1×10^6 U937 cells in the presence or absence of heat-aggregated normal human γ-globulin (AHG) (1 mg/ml). AHG was prepared by heating human γ globulin fraction II (Sigma) (50 mg/ml) in phosphate-buffered saline for 20 min at 63°C and centrifugation for 30 min at 1000*g*. After being washed and subsequently incubated as described above, the culture supernatants were harvested on day 7 for determination of virus yields by p24 ELISA. The results are expressed as mean ± SD of two values, and two-way analysis of variance was used. HIV-1 infection was significantly ($P < 0.005$) enhanced by the diluted antibody-positive serum compared with virus alone, whereas AHG significantly ($P < 0.005$) suppressed the enhancing effect of the serum to the same level as the control of HIV-1 plus AHG.

Treatment	p24 (ng/ml)
HIV	65.2 ± 0.1
HIV + antiserum (10^{-5})	133.4 ± 14.7
HIV + AHG (1 mg/ml)	80.7 ± 1.8
HIV + antiserum + AHG	81.1 ± 4.4

Table 3. Infection enhancement activity in IgG and $F(ab')_2$ from HIV-1 seropositive serum. The purified IgG and IgG-$F(ab')_2$ were serially diluted in phosphate-buffered saline at serum dilution equilvalents from 10^{-3} through 10^{-6}. The estimates of serum dilution equivalents were made on a weight basis and the estimate does not change appreciably if based on a molar basis. Each of these dilutions (1 ml) was mixed with an HIV-1 inoculum (1 ml) containing 1×10^4 $TCID_{50}$, and incubated for 2 hours at 4°C. One million U937 cells were incubated with these mixtures, washed, and then incubated as described before. Culture supernatants were harvested on day 7 for determination of p24. Results are expressed as the mean ± SD of two values. The numbers in parentheses show the enhancement index compared to the control of HIV-1 alone. HIV-1 infection was significantly enhanced by the presence of IgG antibody to HIV-1 diluted at serum equivalents from 10^{-4} to 10^{-6} as compared with the absence of IgG ($P < 0.01$ to $P < 0.005$ by analysis of variance and Scheffe's method of multiple comparisons), and IgG at 10^{-3} and $10^{-3.5}$ significantly reduced virus yields ($P < 0.005$). In contrast, $F(ab')_2$ did not enhance HIV-1 infection at any dilution but suppressed virus yields at dilutions up to 10^{-5} ($P < 0.01$ to $P < 0.005$). The virus titers contained in samples from cells exposed to HIV-1 alone and after mixing with IgG at the 10^{-5} dilution were 160 and 640 $TCID_{50}$ of HIV-1, respectively. The serum dilution equivalent is based on the original concentration of IgG in this serum, which was 15 mg/ml; therefore, the 10^{-4} dilution equivalent represents a 1:10,000 dilution of 15 mg of either IgG or $F(ab')_2$ per milliliter of serum.

Serum dilution equivalent*	p24 (ng/ml)	
	IgG	$F(ab')_2$
0*	96.0 ± 3.6 (1.0)	90.6 ± 1.6 (1.0)
10^{-3}	49.3 ± 1.2 (0.51)	
$10^{-3.5}$	72.7 ± 0.7 (0.76)	35.8 ± 1.3 (0.40)
10^{-4}	115.0 ± 1.5 (1.20)	46.8 ± 0.4 (0.52)
$10^{-4.5}$	174.2 ± 3.3 (1.81)	64.7 ± 2.7 (0.71)
10^{-5}	244.8 ± 6.0† (2.55)	69.4 ± 3.1 (0.77)
$10^{-5.5}$	159.4 ± 0.8 (1.66)	93.1 ± 2.5 (1.03)
10^{-6}	121.1 ± 3.9 (1.26)	81.0 ± 2.4 (0.89)

*HIV alone. †Peak enhancement.

Those observations are different from our results for which we used FcR-bearing monocytic cells and heat-stable IgG antibodies to HIV-1.

The presence of enhancing antibodies in the sera of individuals infected with HIV-1 indicates an immunological respone to HIV that may facilitate HIV-1 infection, instead of protecting the host. Conceptually such infection-enhancing antibodies may be more effective when neutralizing antibodies are absent or consumed by virus excess. Epidemiological investigations of the relation between enhancing and neutralizing antibodies and the clinical outcome of HIV-1 infection may determine the role of these antibodies in the progression of the infection. The presence of enhancing antibodies in HIV-1 infections will require careful consideration concerning the development of appropriate HIV vaccines. Further studies will be necessary to characterize the enhancing antibodies, including analysis of the isotypic distribution, the relevant class of FcR, and their relation to neutralizing antibodies and to the antibodies involved in lysis of virus-infected cells. It is particularly important to identify the immunogenic epitopes that react with the enhancing antibodies.

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Mineralocorticoid Action: Target Tissue Specificity Is Enzyme, Not Receptor, Mediated

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Mineralocorticoid receptors, both when in tissue extracts and when recombinant-derived, have equal affinity for the physiological mineralocorticoid aldosterone and for the glucocorticoids cortisol and corticosterone, which circulate at much higher concentrations than aldosterone. Such receptors are found in physiological mineralocorticoid target tissues (kidney, parotid, and colon) and in nontarget tissues such as hippocampus and heart. In mineralocorticoid target tissues the receptors are selective for aldosterone in vivo because of the presence of the enzyme 11β-hydroxysteroid dehydrogenase, which converts cortisol and corticosterone, but not aldosterone, to their 11-keto analogs. These analogs cannot bind to mineralcorticoid receptors.

THE ADRENAL CORTEX IS THE ONLY source of physiological glucocorticoids (cortisol and corticosterone) and mineralocorticoids (aldosterone). Classically, glucocorticoid receptors have been studied by determining the binding of the synthetic radiolabeled ligands, [^{3}H]dexamethasone and [^{3}H]triamcinolone acetonide, because these ligands have both high affinity for receptors and low affinity for transcortin, the plasma corticosteroid binding globulin. High-affinity sites that bind [^{3}H]aldosterone, the physiological mineralocorticoid, occur in a wide variety of tissues—both classical mineralocorticoid target tissues (for example, kidney, parotid, and colon) and others (for example, hippocampus and heart) (*1*). These sites, in the absence of transcortin, show equal affinity in cytosol preparations for aldosterone, cortisol, and corticosterone (the physiological glucocorticoid in mice and rats) and have been termed type I receptors, to distinguish them from type II classical dexamethasone-binding glucocorticoid receptors. In vivo, however, whereas injected [^{3}H]corticosterone and [^{3}H]aldosterone are similarly taken up and retained in the hippocampus and heart, the classical mineralocorticoid target tissues are selective for aldosterone and show little [^{3}H]corticosterone binding (*2*). The recombinant human renal mineralocorticoid receptor has been expressed and its binding specificity determined; just as for cytosol receptors, the human mineralocorticoid receptor in vitro has equal affinity for aldosterone, corticosterone, and cortisol (*3*). Given this equal receptor affinity and the much higher circulating levels of cortisol or corticosterone than aldosterone, additional mechanisms must operate to allow selective mineralocorticoid action in mineralocorticoid target tissues. From our studies (*2*) comparing in vivo with in vitro binding of [^{3}H]aldosterone and [^{3}H]corticosterone, it appears that this selectivity is the result of the exclusion of injected [^{3}H]corticosterone from receptors in mineralocorticoid target tissues.

Stewart *et al.* have proposed (*4*) a mechanism to account for the hypertension and Na^+ retention in patients with the syndrome of apparent mineralocorticoid excess, a syndrome associated with the congenital absence of the enzyme 11β-hydroxysteroid dehydrogenase (*5*). These patients are unable to convert cortisol to cortisone in their kidneys; therefore they have much higher than normal ratios of urinary cortisol to

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