REPLY

We have read the letter of Dr. Siegenthaler regarding our recent paper by Raza et al [1]. The tone of Dr. Siegenthaler's letter is disappointing, and seems inconsistent with the scientific exchange of ideas. The point made by Dr. Siegenthaler is unclear to us especially because, to a large extent, we are in agreement with Dr. Siegenthaler's own work presented in abstract form [2] slightly before our study appeared in print. The Mr of FABP reported in our paper (14.5 kD) is in agreement with the observations from Dr. Siegenthaler's laboratory (14–15 kD). Together these two studies [1,2] undoubtedly demonstrate the existence of fatty acid–binding protein in the skin, the functionality of which will be an important area of research. We are certain that Dr. Siegenthaler will agree with this.

Dr. Siegenthaler is questioning the methodology employed in our study. The methods used in our study are well accepted and documented by several investigators [3,4] including us. It seems that Dr. Siegenthaler does not like the Lipidex-1000 method for FABP study. However, utilizing similar method, studies have been published from the laboratory of Sorof [3,4]. Our point by point response to Dr. Siegenthaler's concerns are given here.

1) Binding methods performed in our study were based on standard protocols used by us previously, and by many other investigators. We do not feel that every detail of negative data should be included in a published study. However, in the text page 324, we have indicated the lack of saturation of binding when HETE, PGE_1, and PGE_2 were used as ligands. Data about the displacement and competition shown in our publication are explanatory enough for the interpretations we have made. Furthermore, a similar approach was made in a previous paper by one of us [3]. Of course our graph may be considered as not fully saturated but it is certainly nearly saturated. The reason for this may be that at high concentrations of ligands the availability to the proteins may be decreased due to solubility problems. Nonetheless, near saturation, difference in total and non-specific binding (with only tenfold excess), competition and displacement provide evidence for a specific and reversible binding. In our study, ligand specificity was further confirmed by showing that HETE, PGE_1, and PGE_2 do not show any saturation and the binding affinity is linear, which suggests a non-specific association with proteins (page 324, second column of our publication). The methodology for the displacement and competition studies is quite similar to that used in a recent publication from the laboratory of Sorof [4]. The parallel line of specific binding observed in our study is probably due to the linearity in non-specific binding with crude cytosol at a high concentration. Because it is not possible to subtract this value from the total binding (because the insolubility of cold ligands will interfere with the interpretation) the graph was not drawn up to the saturation limit.

The competition experiment between RA and FA (oleic acid) was performed and we have mentioned these in the d) section of the discussion, page 326 of our article [1]. The data showing that RA is not competing with FA and vice versa has not been included in this manuscript, and an explanation for this is given in the text.

Using total epidermal cytosol the apparent (extrapolated from Scatchard analysis) binding was 0.5 pmol/μg protein. This binding by no means reflect binding alone with CRABP as suggested by Dr. Siegenthaler. This is total binding with crude cytosol, which may represent one or several types of proteins. This is clearly stated in the text that the precise nature of binding will be known only after purification. This has not been addressed by our study, and should be an area of further investigation.

2) Dr. Siegenthaler should have read our paper more carefully before making an uncalled for statement. We know the difference between PAGE and SDS-PAGE. We have written under the legend to Fig 5 (line 4 onward) the description of our methods. We have not used SDS to study the binding. In our study, we first allowed the radioactive ligands to bind with the cytosolic proteins under Lipidex-1000 assay conditions and then bound and free ligands were separated and only protein-bound ligand(s) were subjected to SDS-PAGE. In this same legend, it is clearly explained that the binding was performed as in Fig 1. These data are most convincing that epidermal cytosol is a 14–15 kD protein that specifically binds fatty acids as compared to other smaller peaks.

Based on the arguments listed above we feel certain that Dr. Siegenthaler will now join us in accepting the fact that FABP exists in rat skin. The additional functions of this protein in skin will certainly be an interesting area of research.

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Seroreactivity Against HPV 16 E4 and E7 Proteins in Renal Transplant Recipients and Pregnant Women

To the Editor:

A number of studies has shown that immunosuppressed individuals are at an increased risk for both human papillomavirus (HPV) infection and development of HPV-associated benign and malignant diseases [1–5]. Besides facilitating primary HPV infection, decreased immunocompetence may also be involved in reactivation of latent HPV. For example, iatrogenic immunodeficiency after renal transplantation and the transient immunosuppression associated with pregnancy can lead to HPV reactivation [1,2]. Furthermore HPV-associated genital lesions are more aggressive in immunosuppressed patients, probably due to the lowered antiviral defenses [6].

In order to evaluate seroreactivity against HPV 16 in immunosuppressed individuals we tested sera obtained from 121 renal transplant recipents (RTR) [7] and 38 pregnant women in Westernblot-assays using as antigens the HPV 16 gene products E4 and E7 expressed as bacterial fusion proteins [8]. Sera from patients attending the hospital for reasons unrelated to HPV or human immunodeficiency virus (HIV) infections, or to cancer were taken as controls [8]. The history of these patients concerning past or present HPV infection was unknown to us. The tests were performed as described previously [8]. As shown in Table I, anti-HPV 16 E4 and E7 reactivity was increased in RTR as compared to age-matched controls

Table I. Seroreactivity against HPV 16 E4 and E7

Patients	n	Age (years)	E4 Positive (%)	E7 Positive (%)
All RTR	120	26–68	31.7[a]	15.0[c]
RTR with skin cancer	35		28.6[b]	11.4[d]
Controls	215		10.2[a,b]	3.7[c,d]
Pregnant women	38	20–45	13.2 (ns)[e]	2.6 (ns)
Controls	81		12.3 (ns)	3.7 (ns)

[a] $p < 10^{-5}$, odds ratio = 4.68.
[b] $p = 0.004$, odds ratio = 3.18.
[c] $p = 10^{-5}$, odds ratio = 6.37.
[d] $p = 0.04$, odds ratio = 4.38.
[e] ns, not significant.

($p < 10^{-5}$). Antibody positivity was not associated with the duration of immunosuppression (between 8 and 20 years). The occurrence of abnormal genital smears or HPV-associated anogenital lesions in these patients was not assessed. However, a virus-induced cytopathologic effect, which indicates HPV replication, or cervical intraepithelial neoplasia would be expected in about 17% and 10% of the female patients, respectively [3]. Such lesions appear after an average of 40 months of immunosuppression [3]. For immunosuppressed men an elevated risk of developing HPV 16–associated anal intraepithelial lesions was described [4,5]. No association was found between anti-HPV16 E4 and E7 reactivity and the presence of skin cancer. This points to type specificity of the antibodies, because skin cancer biopsies of renal transplant patients have been reported to contain HPV5- or 8-DNA [9].

In sera of 38 pregnant women the prevalence of anti-HPV 16 E4 and E7 antibodies was not higher than in sera of age- and sex-matched controls (see Table I) although HPV 16-DNA in cervical smears and HPV-associated lesions can be demonstrated more often during pregnancy than in nonpregnant women [2]. In this study no clinically detectable genital lesions were diagnosed, but the presence of HPV infection and short-term replication, which are presumably sufficient for induction of an antibody response, cannot be excluded. This result may be comparable to the unchanged antibody status after cytomegalovirus reactivation in pregnant women described by Stagno and co-workers [10]. Ongoing experiments will have to show whether antibodies are produced after the development of a lesion and therefore whether in pregnant women anti-HPV 16 reactivity can be regarded as an indicator of disease. Cellular immunity is probably the most important part of the immune defense against primary infection and reactivation of latent HPV. Our data suggest that anti-HPV 16 E4 and E7 antibodies are indeed unlikely to be effective in preventing the disease, but that they might be useful as markers for viral replication and HPV 16 associated lesions in immunosuppressed patients.

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